# EFFECT OF ANTIFOULING PAINT ON HISTO-HEMATOLOGY OF AFRICAN CATFISH (*Clarias gariepinus*) JUVENILE

*By:*


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# CERTIFICATION

This is to certify that this work was conducted and presented by:

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The report has been read and approved having met the requirements of the Department of Fisheries and Aquaculture, Faculty of Agriculture, Federal University Oye-Ekiti, for the award of Bachelor of Fisheries and Aquaculture degree (B. Fish and Aquaculture).

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EXTERNAL EXAMINER

## DEDICATION

This research work is dedicated to the Almighty God and my wonderful parents.

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To God be the glory for his love and kindness towards me before, during and after this project work. I wish to sincerely appreciate my Supervisor; Mrs. Elesho F.E of the Department of Fisheries and Aquaculture and also Mr. Omobepade B.P of the Department of Fisheries and Aquaculture for their kind words, moral support and encouragement throughout the period of this project work. I pray God will continue to bless you in your endeavours and grant all your heart desires.

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AWOLUMATE OLUWASANMI TOLULOPE

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# ABSTRACT

The study investigated the histo-hematological properties of African Catfish exposed to antifouling paint. Concentrations 0.75ml/L, 1.00ml/L, 1.25ml/L, 1.50ml/L and control which were fractions of a preliminary 96h $LC_{50}$ and control were used in the static bioassay. Physicochemical parameters (Temperature, pH, DO) of the test media were measured across the concentrations and it was found that Temperature and pH remained the same during the 96hours exposure period while Dissolved Oxygen concentration was depleted with increase in concentration and hours. This induced stress on *Clarias gariepinus* and resulted in behavioral changes like air gulping, loss of reflex, rapid opercula movements and death at higher concentrations of 1.00ml/L, 1.25ml/L and 1.50ml/L between 72hours to 96hours exposure. The blood parameters PCV and RBC, and differential counts such as neutrophil decreased with increasing concentration of the toxicant and become significantly lower ($P < 0.05$) at higher concentration when compared with the control. While WBC and Lymphocyte were observed to have increased with increase in concentration of the toxicant. It is believed that observed depression in PCV coupled with decreased and RBC are obvious signs of anemia. Degenerative changes were observed in the organs of *C. gariepinus*. The gills of fish in the varying concentrations particularly the highest concentrations (1.50ml/L) showed signs of severe necrosis with severe inflammation. The fish in highest concentrations also showed degenerative changes such diffuse lobular hepatocytes necrosis with severe inflammation but no fibrosis.

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# CHAPTER ONE

## 1.0 INTRODUCTION

Antifouling paints (AF) containing biocides have caused unwanted environmental consequences both in the past and in the present (Dafforn *et al.*, 2011). Different chemicals from antifouling agent from paint industry contribute a lot to water pollution forming a threat to aquatic plants and animals. In modern times, antifouling paints are formulated with copper, organotin compounds or other biocides, which impede growth of algae and other marine organisms. A greater part of this chemical becomes pollutants which exhibit bio-magnification and bioaccumulation capabilities with a broad spectrum of impacts, and stresses on aquatic organisms (Censi *et al.*, 2006). Copper oxide concentrations exceed water quality standards in several areas (Brooks *et al.*, 2007) and have led to reduction in the number of boats allowed in marinas (Sánchez-Rodríguez, 2011). Elevated copper concentrations in sediment have been shown to reduce biodiversity and biomass in macro benthic communities and cause olfactory dysfunction in fish (Thomas *et al.*, 2010).

Antifouling paints have been used mostly in the coastal cities of Nigeria. Boats, ships at the docks and fishing gear such as nets have been known to have been painted with antifouling paints. Fisher folks make use of different types and brands of antifouling paints which are either imported into the country or manufactured by indigenous paint manufacturers. Fisher folks make use of these paints without knowing the consequence they have on our natural water bodies, aquaculture facility and our fisheries resources. Fish are often exposed to highly contaminated water, especially in areas where the dilution rate of waste water is low. Water pollution can lead to different changes ranging from biochemical alterations in single cells up to changes in whole populations. In the 1990s, the concept of biomarkers with fish as a major biomarker has being increasingly established (Achuba and

Osakwe, 2003; Monterio *et al.*, 2007; Miller *et al.*, 2007; Farombi *et al.*, 2007; Pavlović *et al.*, 2010). According to Huggett et al. (1992), the most common usage of the term biomarker has been for biochemical, physiological or histological indicators of either exposure to or the effects of xenobiotic chemicals at the sub-organismal or organismal level. The use of *Clarias gariepinus* in this study is basically because it is the most farmed fish in Nigeria and because it has a high tolerance level towards pollution in the aquatic ecosystem thus it can be used to measure the rate at which antifouling paint can begin to induce stress and cause damage to fishes

Hematological indices have been employed in effectively monitoring the responses of organisms to stressors and thus its health status under such adverse conditions (Oriakpono *et al.*, 2012). Generally, hematological tests are used to establish normal health status and to diagnose diseases caused by various factors namely heavy metals, environmental stress, parasitic infections, genotoxic effect of pollutants, nutrition, and pollution in human and veterinary science (Fedato *et al.*, 2010). Hematological parameters act as physiological indicators to changing external environments as a result of their relationship with energetic (metabolic levels), respiration (hemoglobin) and defense mechanisms (leukocyte levels) (Caruso *et al.*, 2005).

Histopathological changes in animals tissues are powerful indicators of prior exposure to environment stressors and are net result of adverse biochemical and physiological changes in an organism (Reddy 2013). Histopathological biomarkers can be indicators of the effects on organisms of various anthropogenic pollutants and are a reflection of the overall health of the entire population in the ecosystem (Reddy *et al.,* 2013). Well documented lesions based on experimental data in liver, kidney, gill, ovary, skeletal system and skin have been used as biomarkers to date (Akpolih *et al.*, 2013). Histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the

affected organism (Reddy 2013). Most biomarkers are narrow in their expression whereas histopathology is broad in its evaluation (Medja *et al.*, 2011).

Stress is a general and non-specific response to any factors disturbing homeostasis. Stress reaction involves various physiological changes including alteration in blood composition and immune mechanisms (Svoboda 2001; Witeska 2003; Ololade 2010). It has also been linked to disease outbreaks, low productivity and mortality in aquaculture. This study will state the toxic stress, histopathology and hematological effect of various concentrations of antifouling paints to*Clarias gariepinus* in a juvenile stage of growth which will be carried out within 96hours.

## 1.1 STATEMENT OF RESEARCH PROBLEM

Due to the fact that boats or vessels are packed by mooring them on the water body, the antifouling paint that are used on these boats and vessels have a way of leaching out slowly into the water body over time, as a result harmful chemicals from the paints, mostly heavy metals, are slowly released into the aquatic ecosystem. The impact and effect of these heavy metals on the aquatic ecosystem and organisms therein cannot be over emphasized, more so the issue of bioaccumulation and biomagnification on consumers of aquatic organisms that are harvested from such polluted water body.

Furthermore, effluent released from antifouling paint manufacturing industries can also find its way into the aquatic ecosystem and cause detrimental effect on the fisheries resources. Washing of painting materials near the water body by local fishing boat painters also contribute to high level of pollutants in the aquatic ecosystem.

## 1.2 JUSTIFICATION FOR THE SUDY

In southwestern Nigeria, especially in the coastal areas like Igbokoda in Ondo state and its environs, paints like Mechalin® super automotive car paint is used as an antifouling paint. Fishing gears are also treated with antifouling paints to prevent biofouling of such fishing gears and as such increase the durability of the gears. This gears when used leaches the paint chemicals into the water body. Also, structures built on or submerged in water are treated with, antifouling paint is to prevent biofouling so has to increase the durability of such structures thereby causing more harm to the aquatic environment. These paints when used are thinned with kerosene before it is applied on boats, hulls of ships and fishing gears e.g gill nets. Chemicals from the paints when they dissolve can pose a great threat to the aquatic organisms in the water. Bioaccumulation of these pollutants in fish, for example, when consumed can in turn be detrimental to the health of humans.

Different forms of pollutants have been studied and their effects have been documented but little attention has been drawn towards the response of fish and other aquatic organism to the constant pollution of water bodies by antifouling paints which are used to paint the hull of ships, vessels or boats. Moreover, other sources may also be from the effluents of paint manufacturing companies being released into the water bodies. The research model will be a simulation of what may occur in the natural environment so has to effectively predict the effect of the pollutant on aquatic organism.

Growing human population and industrialization have led to the pollution of most aquatic ecosystems and consequently result in deterioration of the environmental water quality. Indicator organisms are needed to improve assessment programs on the ecological impacts of anthropogenic

activities on the aquatic environment. Fish have been widely documented as useful indicators of environmental water quality because of their differential sensitivity to pollution. This study will investigate the toxic stress and his-hematological changes of *Clarias gariepinus* as biological indicator because this fish has slim tolerance level to heavy metal pollution. Furthermore, the concentration at which each heavy metal causes changes to the fish will be determined and also the heavy metal concentration will be compared with the WHO standard.

This study is also important so as to be able to determine the heavy metal concentration which is present in antifouling paints, their implications on fisheries resources as they affect both the ecosystem and the organisms in the sense that it can disrupt the genetic makeup of the aquatic organism and even have impact on the food chain and food web thereby altering the processes within the aquatic ecosystem, in the same vain affecting fish quality and even reduce the population of fish that it available for capture from polluted water bodies, whereby affecting fish exportation. The toxic stress and hematological effects of various heavy metals such as Copper, lead, mercury and Cadmium on the mortality and behavioral changes and hematology of different fish species have been reported, (Bill *et al.,* 2007) opined that zinc is an essential micro-nutrient often associated with enzymes and proteins but at toxic levels it causes pale and congested gills in *Oreochromis niloticus.* John *et al.* (2007), also stated that livers of channel catfish (*Ictalurus punctatus*) exposed to chlorinated effluents from a wastewater treatment plant were enlarged and showed histological lesions.With these metals, various physiological and biochemical indices in fish havebeen investigated and consequently used in various scientific and ecological studies; (Kori-Siakpere *et al.*, 2006; Maheswaran *et al.* 2008). Usually, red blood cell (RBC) system of fish reacts to heavy metal intoxication with anemia but in some cases, particularly after short exposures, blood parameters (haematocrit, RBC, mean corpuscular volume, hemoglobin) may be increased (Dethloff 1999, Ahmad *et al.,* 2015). However,

not much work has been carried out on the effect of antifouling paint on the toxic stress and histo-hematology of *Clarias gariepinus*.

This study will be to evaluate selected histological and hematological effects resulting from the exposure of the freshwater fish, *Clarias gariepinus* to sub lethal concentrations of antifouling paint in water.

## 1.3 RESEARCH OBJECTIVES

The general objective of this research is to assess the toxic stress of antifouling paint on *Clarias gariepinus*, the specific objectives are to:

- assess the mortality rate of *Clarias gariepinus* exposed to antifouling paint;
- assess the behavioral changes of *Clarias gariepinus* exposed to antifouling paint;
- assess the morphological changes of *Clarias gariepinus* exposed to antifouling paint;
- evaluate the heavy metal concentration of *Clarias gariepinus* exposed to antifouling paint;
- evaluate the hematological properties of *Clarias gariepinus* exposed to antifouling paint;
- evaluate the histological properties of *Clarias gariepinus* exposed to antifouling paint; and
- evaluate the differential toxicity ($Lc_{50}$) of selected antifouling paint to *Clarias gariepinus*.

## 1.4 RESEARCH HYPOTHESIS

$Ho_1$ – there is no significant difference on the mortality rate of *Clarias gariepinus* exposed to antifouling paint,

$Ha_1$ – there is significant difference on the mortality rate of *Clarias gariepinus* exposed to antifouling paint,

$Ho_2$ – there is no significant difference in the behavior of *Clarias gariepinus* exposed to antifouling paint,

$Ha_2$ – there is significant difference in the behavior of *Clarias gariepinus* exposed to antifouling paint,

$Ho_3$ – there is no significant difference in the morphology of *Clarias gariepinus* exposed to antifouling paint,

$Ha_3$– there is significant difference in the morphology of *Clarias gariepinus* exposed to antifouling paint,

$Ho_4$ – there is no significant difference in the heavy metal concentration of *Clarias gariepinus* exposed to antifouling paint,

$Ha_4$ – there is significant difference in the heavy metal concentration of *Clarias gariepinus* exposed to antifouling paint,

$Ho_5$– there is no significant difference in the hematological properties of *Clarias gariepinus* exposed to antifouling paint,

$Ha_5$ – there is significant difference in the hematological properties of *Clarias gariepinus* exposed to antifouling paint,

$Ho_6$ – there is no significant difference in the histological properties of *Clarias gariepinus* exposed to antifouling paint,

$Ha_6$ – there is significant difference in the histological properties of *Clarias gariepinus* exposed to antifouling paint,

**$Ho_7$– the differential** toxicity ($Lc_{50}$) of antifouling paint on *Clarias gariepinus* does not differ **significantly,**

**$Ha_7$– the** differential toxicity ($Lc_{50}$) of antifouling paint on *Clarias gariepinus* does differ **significantly.**

# CHAPTER TWO

# LITERATURE REVIEW

## 2.0Taxonomyand Biology of the African catfish, *Clarias gariepinus*

African catfish (*Clarias gariepinus*) is a species of catfish of the family Clariidae, the air-breathing catfishes. Catfish belongs to the kingdom Animalia, phylum Chordata, Superclass Osteichthyses, class Actinopterygii, subclass Neopterygii, infraclass Teleostei, superorder Ostariopysi and order Siluriformes. They are found throughout Africa and the Middle East, and live in freshwater lakes, rivers, and swamps, as well as human-made habitats, such as oxidation ponds or even urban sewage systems_(Froese *et al.*, 2014).

### 2.0.1 Description

The African sharp-tooth catfish is a large, eel-like fish, usually of dark gray or black coloration on the back, fading to a white belly. *Clarias gariepinus* has an average adult length of 1m–1.5 m (3 ft 3 in–4 ft 11 in) (Froese *et al.,* 2014). It reaches a maximum length of 1.7 m (5 ft 7 in) TL and can weigh up to and more than 60 kg (130 lb).These fish have slender bodies, flat bony heads, notably flatter than in the genus *Silurus*, and broad, terminal mouths with four pairs of barbels. They also have large accessory breathing organs composed of modified gill arches. Also, only the pectoral fins have spin (Froese *et al.,* 2014).

### 2.0.2 Habits

It is a nocturnal fish like many catfish. It feeds on living, as well as dead, animal matter. Because of its wide mouth, it is able to swallow relatively large prey whole (Anoop *et al.,* 2009). It is

also able to crawl on dry ground to escape drying pools. Furthermore, it is able to survive in shallow mud for long periods of time, between rainy seasons.

### 2.0.3 Habitat and Biology

This species is found in lakes, streams, rivers, swamps and floodplains, many of which are subject to seasonal drying. The most common habitats are floodplain swamps and pools where they can survive during the dry season(s) due to their accessory air breathing organs (Froese *et al.*, 2014). *Clarias gariepinus* undertake lateral migrations from the larger water bodies, in which they feed and mature at about the age of 12 months, to temporarily floody marginal areas in order to breed. These reproductive migrations typically take place shortly after the onset of the rainy season(s). The final gonadal maturation is associated with rising water levels. Under stable environmental conditions, adult *C. gariepinus* have mature gonads year-round. Under ideal conditions, a ripe female may lay about 60 000 eggs/kg. Prior to mating, males compete aggressively for females with which they mate in single pairs, the female swishing her tail vigorously to mix the eggs and sperm and distribute the fertilized eggs. The adhesive eggs stick to submerged vegetation and hatch in 20–60 hours, depending on temperature. The yolk sac is absorbed within 3–4 days and the stomach is fully functional within 5–6 days after onset of exogenous feeding. Sexual differentiation begins between 10 and 15 days after hatching. Larvae feed and grow rapidly in the warm (usually >24 °C) nutrient rich floodplains, reaching 3-7 g within 30 days. As flooded marginal areas dry up with the end of the rains, juveniles and adults make their way back to deeper water. In areas with two rainy seasons, there are usually two reproductive peaks during the year, corresponding in intensity to the magnitude of the rains (Anoop *et al.*, 2009).

## 2.1 ANTIFOULING PAINT

The Antifouling Paint used was Mechalin Automotive Paint and it is a copper based antifouling paint. This antifouling paint is used for treatment of cage nets and fish gears (e.g. fishing nets) in aquaculture and also for painting boats and hulls of ships in capture fisheries. The paint contains a latex matrix which contains 43% cuprous oxide ($Cu_2O$) as the active ingredient (22% metallic copper). It also contains Chlorothalonil (2,4,5,6- terachlorioisopthalonitrile) which is a booster biocide for the control of copper resistant fouling organisms such as algal slimes and zinc pyrithione.

### 2.1.1 BIOFOULING

Marine biofouling is the undesired attachment and growth of microorganisms, plants and animals on submerged surfaces in the aquatic environment. Immediately after submerging a surface in the marine environment it attracts organic particles, proteins glycoproteins, creating an initial organic film that attracts the primary colonizers bacteria and diatoms (Cecilia Ohlauson, 2013). Within a week colonization occurs with spores of macro algae, protozoa and following them are the larvas of marine invertebrates (Yebra *et al.,* 2004). The composition of fouling organisms on a submerged surface varies around the world and is largely influenced by water characteristics such as temperature, salinity and pH which also regulate the amount of fouling generated. This diverse fouling community can in some environments increase the hull friction of a ship up to 0.5 % per day affecting both maneuverability of the vessel and the fuel consumption which could be increased by up to 40% during a period of six months (WHOI 1952, Schultz *et al*., 2011).

The increase in fuel consumption can be avoided with antifouling techniques such as antifouling paint (Finnie and Williams 2010). Another problem with biofouling is the transportation

of invasive species around the world which harm aquatic ecosystems (Mackie *et al.*, 2004, IMO 2011).

### 2.1.2 HISTORY OF ANTIFOULING PAINTS

Antifouling methods are not modern inventions only, they have been used since ancient times to protect ship hulls, starting with tar, asphalt and wax derived from nature. Around 700 B.C. it is thought that the use of copper as an antifoulant first started with sheathings on ship hulls, an idea that was in use on and off until the 18th century. In the mid-19th century the first real antifouling paints based on linseed oil, rosin or shellac came in use with copper, arsenic or mercury oxides as biocide. These paints were effective against fouling and were in use until the late 1940s when health and safety concerns about arsenic and mercury were raised, which made copper-based paints the most popular (Readman 2006, Almeida *et al.*, 2007) of all. In the end of the 1950s a new biocide came in to the antifouling market, tributyltin (TBT), with outstanding antifouling efficiency. The combination of TBT oxide with self-polishing copolymer (SPC) formulations in the 1970s gave an antifouling paint with constant leaching rates, longer service life and resulted in exceptionally smooth ship hulls. By the middle of the 1980s 80% of the commercial fleet were painted with TBT-SPC paint (Abbott *et al.*, 2000). However, the TBT was released into the water and contaminated harbors and coastal areas (Fent, 1996). By 1980, negative aspects of TBT usage became noticeable, first in France with shell abnormalities in oysters, and then in England with development of male genitalia in female gastropods (imposex) causing reproductive failure (Abbott et al. 2000). France was also the first country to enforce limitations on TBT usage, followed by England and most industrialized countries in the 1980s (Champ 2000). In 1999 the International Maritime Organization (IMO) adopted a convention prohibiting all application of TBT-containing Antifoulant on ships by 1st of January 2003 15 and total prohibition by 1st of January 2008. The convention would however not come into force

immediately. It required that 25 states, representing 25% of the world's merchant shipping tonnage, consented (Champ 2003). This was achieved on the 17th of September in 2008 when the convention thus came into force (IMO 2008). Following the TBT ban, self-polishing copolymer paints (SPC) and controlled depletion paints are still the most used technologies to protect ship hulls from fouling (Almeida *et al.*, 2007). The SPC are based on an acrylic polymer matrix with pendant groups, usually copper or zinc but also organosilicons in the form of silyl (Finnie and Williams 2010). The pendant groups and additional co-biocides in the paint are released through hydrolysis or ion exchange which is followed by erosion of the paint layer.

Controlled depletion paints (ablative/erodible paints) are in general based on a water soluble binder combined with metallic pigments and polymers to control erosion of the paint. The biocides are released at a constant rate together with the soluble binder and can therefore be better controlled than in self polishing paints. One drawback is however that the controlled depletion paints need a higher biocide concentration to maintain efficacy (Almeida *et al.*, 2007).

Copper oxide is the main antifouling biocide used today (Thomas and Brooks 2010). Questions have however been raised about the environmental consequences of Cu ions since high levels in the environment have been reported for areas with large boating activity (Singhasemanon *et al.*, 2009). A ban or phase out has been discussed in two states in USA (Carson *et al.*, 2009, Prichard 2010, Senate Bill 5436: Prohibiting copper in antifouling paints used on recreational water vessels 2011). Before the TBT ban, copper and TBT was used in combination with high efficacy. To fill the gap after TBT, several new co- biocides were developed (Hellio 2010). The most common co-biocides in use today are chlorothalonile, copper pyrithione, dichlofluanide, tralopyril, cybutryne, tolylfluanide, DCOIT, zinc pyrithione and zineb.

### 2.1.3 ENVIRONMENTAL IMPACT OF ANTIFOULING PAINT

Antifouling methods can be divided into chemically acting and physically acting. The chemically acting methods reduce fouling through the release of a biocide, which is defined as a substance that is intended to control the effect of harmful organisms with a chemical or biological mode of action (EU 1998). The physically acting methods reduce fouling with physical properties such as hydrophilic or hydrophobic surfaces (Buskens *et al.*, 2012), ultrasound (Guo *et al.*, 2011), oxygen-free layers (Lindgren *et al.*, 2009).

### 2.1.4 Categories of Antifouling Paints

There are three main categories of antifouling paints:

a. **Conventional** or referred to as "free association," in which the biocides are loose in the paint and are released by contact leaching;
b. **Soluble matrix** and ablative; and
c. **Self-polishing**, in which the biocides are added in free association or chemically integrated within a matrix as in the organotin copolymer paints.

Categories (a) and (b) are referred to as conventional paints; both use the biocide in the free association form. Category (a) ("free association") uses contact leaching to release the biocide. In this process, seawater percolates slowly through a tough insoluble paint matrix. The biocides are added in the free association form and are mixed into the paint. They leach exponentially with time. This category of TBT antifouling coatings has traditionally posed a problem of high early release rate with subsequently shortened time period of protection from attachment and growth of fouling organisms. After a period of less than 2 years, the paint film ages, calcium carbonate ($CaCO_3$) clogs the micro channels in the paint surface and inhibits the release of biocide, then the surface becomes

biofouled. This leaves a quantity of biocide that remains unused on the vessel hull, which must be removed prior to the next painting. The removed paint film must be properly disposed of or it may then be a source of environmental contamination.

There may be a significant amount of TBT remaining in the film when the fouled film is removed. There have been few studies directed to the extent to which the old paint film is a source of TBT to the environment.

**Category (b)** is commonly referred to as an "ablative" (or shedding) paint. It is a slightly seawater-soluble matrix paint that sheds during use as the paint surface roughens, paint particles (very thin microlayers) peel off, and exposing a fresh supply of biocide. The lifetime of this paint is about 2 years.

**Category (c)** antifouling paint is commonly referred to as "self-polishing" copolymer paint. Developed in the early 1970's, the paint is hydrophobic (i.e., seawater does not enter into the paint matrix). The seawater/paint reaction layer occurs at the surface of the paint; the paint has an unstable release layer that gradually erodes.

### 2.1.5 EFFECT OF CHEMICALS USED AS BIOCIDE IN ANTIFOULING PAINT ON AQUATIC ORGANISMS

#### 2.1.5.1 Copper Oxide

Copper is an essential metal. However, although it is an effective biocide, it may also affect non target organisms and cause environmental concerns (Kiaune *et al.*, 2011). The toxicity of copper in water is greatly affected by the chemical form or speciation of the copper and to what degree it is bound to various ligands that may be in the water, making the copper unavailable to organisms

(Burridge *et al.*, 2010). The speciation is essential for understanding the copper's bioavailability and subsequent toxicity to aquatic organisms (Thomas *et al.*, 2010). Copper oxide when used in antifouling paint leaches from the boat surfaces and enters the water as a free copper ion ($Cu^{+}$ ), which is immediately oxidised to $Cu^{2+}$ and forms complexes with inorganic and organic ligands (Thomas *et al.*, 2010). Copper is a trace element needed at miniscule levels for the proper functioning of all organisms (Kiaune *et al.*, 2011). However, it can be toxic at higher concentrations (Burridge *et al.*, 2010). Copper is generally toxic to aquatic organisms, with a lethal concentration 50 ($LC_{50}$) value varying from 5 to 100,000 µg L−1 (USEPA 1995). However, organisms have different mechanisms by which they cope with and process copper (Thomas *et al.*, 2010). Generally copper is actively regulated in fish, decapod crustaceans and algae. It is stored in bivalves, barnacles and aquatic insects (Brix *et al.*, 2000). The bioavailability, bio-distribution to various parts of the organism and bioaccumulation of copper are dramatically influenced by water chemistry. Therefore, water pH, hardness, organic content and salinity play important roles in copper-induced toxicity (Burridge *et al.*, 2010).Thus, increased pH accentuates copper toxicity because of the reduced competition between copper and hydrogen ions at the cell surface (Wilde *et al.*, 2006). In a similar manner, cations that are involved in water hardness also compete with Cu2+ for biological binding sites (Boulanger *et al.*, 2003). Copper bound to organic matter is widely thought to be non-bioavailable and, therefore, non-toxic (Brooks *et al.*, 2007). Dissolved organic carbon (DOC) content is among the most important factors in reducing copper toxicity in both fresh- and salt-water species (Kiaune *et al.*, 2011). DOC forms organic complexes with copper, thereby reducing copper's bioavailability (Kiaune *et al.*, 2011). The effects of DOC on reducing the toxicity of copper have been reported in fish (Playle *et al.*, 1993), bivalves, (Brooks *et al.*, 2007), echinoderms (Lorenzo *et al.*, 2006), macroalgae (Brooks *et al.*, 2008), unicellular algae (Florence *et al.*, 1986), estuarine copepod

(Hall *et al.*, 2008) and planktonic crustaceans (Kramer *et al.*, 2004). Some authors confirm that water salinity influences the bio-distribution and bioaccumulation of copper, affecting its toxicity (Polo *et al.*, 2011).

### 2.1.5.2 Chorothalonil

Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) is a pesticide used widely in agriculture, silviculture and urban settings. This pesticide can enter surface waters through rainfall runoff, spray drift or atmospheric deposition, subsequently impacting aquatic biota (USEPA 1999). It is used as a booster biocide in marine paints as one of the chemicals replacing the widely banned organotin fungicides, such as tributyltin, resulting in greater potential for Chlorothalonil contamination of marine waters and sediments (Voulvoulis *et al.*, 2000). Chlorothalonil is a broad-spectrum fungicide with a Kow of 2.64–4.28 and a water solubility of 0.9 mg L−1 (Caux *et al.,* 1996). Chlorothalonil can be acutely toxic (50% lethal concentration, $LC_{50}$) to fish following 96 h exposures ranging from 8.2 to 76 μg L−1 , depending on the species and the exposure conditions (Davies *et al.*, 1994). Chlorothalonil can accumulate in the tissue of fish. Bioaccumulation factors have been reported to be 18 for willow shiner (*Gnathopogon caerulescens*) and 25 for carp (*Cyprinus carpio*) following sublethal exposures (1.1–1.4 μg L−1) (Tsuda*et al.*, 1992). It has been suggested that leukocytes may be a potential target of toxicity because significant decreases in leukocyte values were found in the Australian freshwater fish Pseudaphritis urvulii, which was exposed for 10 d to 4.4 μg L−1 chlorothalonil (Davies *et al.*, 1994). In vitro studies have demonstrated that the exposure of fish (*Morone saxatilus*) macrophages and oyster hemocytes to chlorothalonil (10 ± 500 μg L−1 ) suppressed immune-stimulated ROS (reactive oxygen species) and baseline NADPH (nicotinamide adenine dinucleotide phosphate) concentration but did not inhibit phagocytosis (Baier-Anderson *et al.*, 2000).There are numerous toxicity studies for chlorothalonil on marine animals, such as

crustaceans (USEPA, 2000), molluscs (Ernst., *et al* 1991), tunicates (Bellas J, 2006) and teleosts (Davies *et al.*, 1994).

#### 2.1.5.3 **Dichlofluanid**

Dichlofluanid (N-dichlorofluoromethylthio-N0-dimethyl-N-phenylsulphamide) has been commonly used as herbicide on crops (Lee *et al.,* 2010). Dichlofluanid has a lower toxicity compared with other Antifouling agents, although some studies have identified its toxic effects (Lee *et al.,* 2011), such as embryotoxicity in sea urchin, *Glyptocidaris crenularis* (Xu *et al.*, 2011).

#### 2.1.5.4 **DCOIT (Sea Nine 211®)**

One of the new alternative biocides is 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT), the active ingredient of the Sea Nine 211® Antifouling Agent manufactured by Rohm and Haas Company (Steen *et al.,* 2004). Aquatic microcosm and marine sediment studies demonstrate that the predominant route of DCOIT dissipation in the marine environment is its rapid biodegradation (Steen *et al.,* 2004). DCOIT predominantly undergoes biotic degradation under both aerobic and anaerobic conditions with biological degradation over 200 times faster than hydrolysis or photolysis (Thomas *et al.,* 2010). Biodegradation is a very effective mechanism for the detoxification of the compound since the resulting metabolites are five orders of magnitude less toxic than the parent compound (Jacobson *et al.,* 2000). However, Sea-Nine antifouling agent is acutely toxic to a wide range of aquatic organisms although no chronic toxicological effects have been observed in the extensive toxicology tests conducted on it (Shade *et al.,* 1994). DCOIT has a log KOW of 2.8 and an aqueous solubility of 14 mg L−1 (Thomas *et al.,* 2010). There are numerous studies that have investigated the toxicity and effects of DCOIT on marine animals. These studies demonstrated the following: larval mortality in crustaceans (Jacobson *et al.*, 2000) embryolarva immobility and embryotoxicity in molluscs (Bellas

*et al*., 2006) embryotoxicity in echinoderms (Kobayashi *et al*., 2002), embryotoxicity and inhibition of larval settlement in tunicates (Bellas *et al*., 2006) and mortality in teleosts (Cima *et al*., 2008).

### 2.3.5.5 **Diuron**

Diuron (1-(3,4-dichlorophenyl)-3,3-dimethylurea) also persists in seawater, but it is less persistent in marine sediments with a half-life of 14 days (Thomas *et al*., 2002). Diuron is relatively soluble in water (35 mg $L^{-1}$) and has a reported log KOW of 2.8 (Thomas *et al*., 2010). Diuron is present at high concentrations in marine surface waters but it has only been detected at low concentrations in sediments (Lamoree *et al*., 2002). Diuron is persistent in the marine environment and partitions poorly between water and sediments. It can remain suspended and available for uptake by marine organisms (Okamura *et al*., 2002). While the toxic effect of the antifouling herbicide diuron to the photosynthetic aquatic biota has been widely studied, its sublethal effects on the different life stages of fish have been under-reported (Gagnon *et al*., 2009). Diuron has been proven to be very toxic for the reproduction of the green freshwater alga *Scenedesmus vacuolatus* (Backhaus *et al*., 2004). It has also been proven to affect planktonic and periphytic microalgae by reducing the chlorophyll levels (Perschbacher *et al*., 2004). Moreover, it has been proven to be toxic to certain bacterial species (Tixier *et al*., 2001).

### 2.1.5.6 **Irgarol-1051**

Irgarol-1051 (2-methylthio-4-terbutylamino-6-cyclopropylamino-s-triazine) is a slightly soluble and moderately lipophilic triazine herbicide used together with copper to control fouling on boat hulls (Hall *et al.,* 2009). Irgarol inhibits electron transport in the photosystem II (PSII) (Holt *et al.,* 1993) by binding to the D1 protein. Irgarol may affect non-target photosynthetic organisms, such as phytoplankton, periphyton and aquatic macrophytes (Hall *et al*., 1999) when leaching into the

marine environment (Dahl *et al.*, 1996). Only a few studies have addressed the possible effect of Irgarol on marine non-target algae (Buma *et al.*, 2009). The effect of Irgarol on green alga *Dunaliella tertiolecta* (Gatidou *et al.*, 2003), *Synechococcus* sp and *Emiliania huxleyi* (Devilla *et al.*, 2005) in natural phytoplankton communities (Readman *et al.*, 2004) periphyton colonization and phytoplankton species has been investigated and the results showed a decrease in growth, inhibition in cell number and a decrease in the photosynthetic activity of these organism-s. These effects have been seen in many different marine plants and algae, such as the eelgrass; Zostera marina (Chesworth *et al.*, 2004) the brown macroalga, Fucus serratus, the green macroalga. Enteromorpha intestinalis, (Tolhurst *et al.,* 2007) and the green macroalga, Ulva intestinalis (Menin *et al.*, 2008).

2.1.5.7 **TCMS Pyridine**

TCMS (2,3,5,6-tetrachloro-4-methylsulphonyl pyridine), which was used in both the textile and leather industries, is one of the more recent Antifouling compounds introduced to the market . (Bragadin *et al.,* 2007). The toxicity of TCMS towards living organisms has already been evidenced (Konstantinou *et al.*, 2004) and substantiated in in vitro studies (Sabev *et al.*, 2004). TCMS has been found to cause immunotoxic effects at concentrations higher than 10 µm in haemocyte cultures of the colonial ascidian *(Botryllus schlosseri)*, causing oxidative stress in the process (Menin *et al.*, 2005).Both diuron and TCMS pyridine exerted immunosuppressant effects on the *(Botryllus spp)* hemocytes when used at concentrations higher than 250 µm and 10 µm, respectively, causing (i) deep changes in the cytoskeleton that irreversibly affect cell morphology and phagocytosis; (ii) induction of DNA damage; and (iii) leakage of oxidative and hydrolytic enzymes due to membrane alteration. Unlike organotin compounds, diuron and TCMS pyridine do not inhibit cytochrome-c-oxidase and only TCMS pyridine triggers oxidative stress.

2.1.5.8 **Zinc Pyrithione**

Zinc pyrithione (ZnPT) (bis(1hydroxy-2(1H)-pyridethionato-o,s)-(T-4)zinc), one of the most popular surrogate Antifouling biocides, has long been widely used as algaecide, bactericide and fungicide (Bao *et al.,* 2008). ZnPT was found to be highly toxic to aquatic plants and animals (Turley *et al.*, 2000) but it was assumed to be environmentally neutral because it could easily photo-degrade to less toxic compounds (Turley *et al.*, 2005) ZnPT is toxic to Japanese medaka fish (*Oryzias latipes*) and also causes teratogenic effects, such as spinal cord deformities in embryos and on the larvae of zebra fish (Danio rerio) (Goka et al., 1994) at very low sublethal concentrations. (Sánchez-Bayo *et al.,* 2005). However, there is a lack of data on the toxicity of ZnPT (Bao *et al.*, 2008).

| chlorothalonile | 2,4,5,6-tetrachloro-isophthalonitrile<br>CAS: 1897-45-6 | | Inhibits thiol-containing enzymes, causing depletion of glutathione reserves leading to oxidative stress. Disturbs ATP production (Cima et al. 2008). |
|---|---|---|---|
| copper pyrithione (Copper Omadine®) | copper 2-pyridinethiol-1-oxide<br>CAS: 154592-20-8 | | Disrupts proton gradients over cell membranes (Al-Adham et al. 1998). |
| cybutryne (Irgarol 1051) | 2-methylthio-4-tertbutylamino-6-cyclopropylamino-s-triazine<br>CAS:28159-98-0 | | Photosystem II inhibitor, blocking of electron transport in the D1 protein (Hall et al. 1999). |
| DCOIT (Sea-nine 211™) | 4,5-dichloro-2-octyl-3(2H)isothiazolone<br>CAS: 64359-81-5 | | Disruption of metabolic pathways by inhibition of dehydrogenase enzymes, consumption of glutathione reserves, inhibiting respiration and ATP synthesis (Williams 2007). |
| dichlofluanide (Preventol A4-S™) | N,N-dimethyl-N'-phenylsulfamide<br>CAS: 1085-98-9 | | Inhibits thiol-containing enzymes by forming disulfide bridges. Inhibits mitochondrial $Ca^{2+}$ accumulation (Hertel et al. 1981). |
| medetomidine (Selektope™) | 4-[1-(2,3-dimethyl phenyl)ethyl]-1-H-imidazole<br>CAS:86347-14-0 | | Stimulation of the octopamine receptor in invertebrates causing hyperactivity (Lind et al. 2010) $\alpha_2$-adrenoreceptor agonist in vertebrates (Scheinin et al. 1989). |
| tolylfluanide (Preventol A5-S™) | N-dichlorofluoromethyl thio-N',N'-dimethyl-N-p-tolylsulfamide<br>CAS: 731-27-1 | | See dichlofluanide. |
| tralopyril (Econea™) | 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile<br>CAS: 122454-29-9 | | Thought to uncouple oxidative phosphorylation in mitochondria, resulting in disruption of ATP production. Based on information regarding closely related biocide chlorfenapyr (Rand 2004). |
| zinc pyrithione (Zinc Omadine™) | zinc 2-pyridinethiol -1-oxide<br>CAS: 13463-41-7 | | See copper pyrithione. |
| zineb | zinc ethane-1,2-diylbis(dithiocarbamate)<br>CAS: 12122-67-7 | | Disrupts aminoacids preventing protein and enzyme production. Multisite inhibitors (Isaac 1999). |

Figure 1: Antifouling biocides and their structures

(Source: Cecilia Ohlauson, 2013)

## 2.2.0 Organotin Compounds

Organotin compounds are now one of the most studied groups of organometallic chemicals, in terms of industrial and agricultural uses and applications. The first applied use was as a mothproofing agent in 1925. In the early 1960's, two organotin compounds (tributyltin oxide (TBTO) and tributyltin fluoride (TBTF)) were first used as a molluscicides to kill several species of freshwater snails that were the intermediate hosts of the worms of the genus *Schistosoma*, which transmit the disease *Schistosomiasis* to humans. This immediately led to the use of tributyltin (TBT) moiety as a paint additive in 1961 for its biocidal properties in antifouling boat bottom paints. Tributyltin compounds used in antifouling paints are chemically characterized by a tin (Sn) atom covalently bonded to three butyl (C^Hg-) moieties. The toxicity of organotin compounds to aquatic organisms is thought to increase with the number of butyl substituents from one to three, and then to decrease with the addition of a fourth butyl group. In order to assess the fate of a particular tributyltin to derivation in water, one must consider the dissociated active form, the TBT cation (Bu^Sn), and its major metabolites presumably formed by progressive debutylation to inorganic tin (Brinckman, 1981). Elemental or inorganic forms of tin (as in mineral deposits or tin can) appear to cause negligible toxicological effects in humans or wildlife. However, in contrast, the TBT's display an increased fat solubility and, consequently, enhanced ability to penetrate biological membranes, thereby posing a greater toxicity potential.

### 2.2.1 EFFECT OF ORGANOTIN COMPOUNDS ON AQUATIC ORGANISM

Experiments with organotin compounds have shown various toxic effects in experimental animals including effects on the immune system, the endocrine system and the liver. It is well known that gastropods, such as the dogwhelk (*Nucellalapillus*), bioaccumulate TBT and its endocrine

disruptive effects in female snails result in the development of structures typical of the male reproductive system like a vas deferens and a penis-homologue ("imposex") (Matthiessen and Gibbs, 1998).

Immune parameters can be valuable biomarkers of effects of xenobiotics substances in fish since the immune system has been shown to be sensitive to the effects of pollution (Anderson and Zeeman, 1995; Vos *et al.*, 1996), and organotin compounds affect the immune system in particular. Also a reduction of the non-specific cytotoxic cell (NCC) activity in European flounder (Grinwis *et al.,* 1998) and channel catfish (*Ictalurus punctatus*) (Rice *et al.*, 1995). Exposure to TBT also caused histopathological lesions in liver, kidney, eye and gill epithelium in medaka (*Oryzias latipes*) and guppy (*Poecilia reticulata*) (Wester and Canton, 1987; Wester *et al.*, 1990), and masculinization in Japanese flounder *Paralichthy solivaceus* (Shimasaki *et al.*, 2003). TBT levels have been found to reach 1.58 pg/litre in sea water and estuaries, 7.1 pg/litre in fresh water, 26 300 pg/kg in coastal sediments, 3700 pg/kg in freshwater sediments, 6.39 mg/kg in bivalves, 1.92 mg/kg in gastropods, and 1mg/kg in fish. However, these maximum concentrations of TBT should not be taken as representative, because a number of factors may give rise to anomalously high values (e.g., paint particles in water and sediment samples).

### 2.2.2 HEAVY METALS IN ANTIFOULING PAINT AND THEIR EFFECT ON ORGANISMS

Antifouling paints are also made using other heavy metals which have detrimental effect on the aquatic ecosystem in the long run. Heavy metals such as mercury, cadmium, copper, lead and nickel are of the most important pollutants which effect aquatic environment and fish. Studies from the field and laboratory works showed that accumulation of heavy metals in a tissue is mainly dependent on water concentrations of metals and exposure period; although some other

environmental factors such as water temperature, oxygen concentration, pH, hardness, salinity, alkalinity and dissolved organic carbon may affect and play significant roles in metal's accumulation and toxicity to fish.

**Aluminum (Al)**

Aluminum (Al) is the third most common and abundant metal on earth after oxygen and silicon (Authman, 2011). It is similar to many other metals in that it is generally considered most toxic in its soluble ionic form (Walton RC et al., 2011). The toxicity of aluminum to fish depends to a large extent on the physicochemical properties of the water and particularly on its pH. Aluminum is soluble at pH values below 6.0 (Svobodová Z, 1993). The mechanism of toxicity in fish seems to be related to interference with ionic and osmotic balance and with respiratory problems resulting from coagulation of mucous on the gills of fish and has been found to cause severe fusion of lamellae and filaments in the gills (Abdel-Latif, 2008). Al is considered to be an endocrine disrupting Chemical in mature *Oreochromis niloticus* females Correia *et al*., 2010). Fish exposed to Al showed significantly higher total erythrocyte counts; haematocrit (Hct); mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) while mean corpuscular volume (MCV) was significantly lower (Alwan et al., 2009).

**Arsenic (As)**

Arsenic reach aquatic ecosystems by a variety of sources including manufacturing companies, mineral or strip mines, smelting operations, and electric generating stations (power plants). One major agricultural source of as is the manufacture and use of arsenical defoliants and pesticides. It also has been used to kill aquatic plants to reduce the difficulty encountered during hook-and-line fishing of areas overgrown with aquatic vegetation (Sorensen, 1991). Arsenic is able to accumulate in

large quantities in the sediments on the bed of water courses and reservoirs, and in aquatic organisms (Svobodová Z, 1993). Arsenic is actively metabolized in the tissue of fish especially in organs such as the liver and has the tendency to accumulate as reported in different teleosts such as green sunfish (Sorensen *et al.*, 1979), rainbow trout (Cockell *et al.*, 1991), Japanese medaka and *Tilapia mossambica* (Suhendrayatna *et al.*, 2002). Donohue and Abernathy (1999) reported that total arsenic in marine fish, shellfish, and freshwater fish tissues ranged from 0.19 to 65, 0.2 to 125.9, and 0.007 to 1.46 μg g−1 dw, respectively. Acute exposures can result in immediate death because of As-induced increases in mucus production, causing suffocation, or direct detrimental effects on the gill epithelium. Chronic exposures can result in the accumulation of the metalloid to toxic levels and is responsible for several disease conditions (Hughes, 2002).

**Cadmium (Cd)**

Cadmium is a naturally occurring nonessential trace element and its' tendency to bio accumulate in living organisms often in hazardous levels, raises environmental concern (Sfakianakis *et al.*, 2015). Cadmium production, consumption and emissions to the environment have increased dramatically during the 20th century, due to its industrial use (batteries, electroplating, plastic stabilizers, pigment), and consequently lead to contamination of aquatic habitats (Järup, 2003). This heavy metal has been shown to accumulate mainly (about 75 %) in kidney, liver and gills of freshwater fish (Chowdhury *et al.*, 2006), but it can also be deposited in the hearts (Cattani *et al.*, 1996) and other tissues (Melgar *et al.*, 1997) and cause pathological changes of varying severity in above mentioned organs (Thophon *et al.*, 2004). Morphological and histological alterations in liver of fishes exposed to cadmium have been documented (Thophon *et al.*, 2003). Higher doses of cadmium caused visible external lesions such as discoloration and necrosis on livers of *Cyprinus carpio*, *Carassius auratus* and *Corydoras paleatus* (Cavas *et al.*, 2005). Omer *et al.*, (2012) reported

**histopathological alterations in liver, intestine and kidneys of tilapia fish (*Oreochromis niloticus*) exposed to cadmium.**

**Copper (Cu)**

Copper (Cu) is an essential trace metal and micronutrient for cellular metabolism in living organisms on account of being a key constituent of metabolic enzymes (Monteiro *et al.*, 2009). However it can be extremely toxic to intracellular mechanisms in aquatic animals at high concentrations which exceed normal levels (Abdel-Tawwab *et al.*, 2007). Fish can accumulate copper via diet or ambient exposure (Sfakianakis *et al.,* 2015). Even at low environmental concentrations, copper shows distinct affinity to accumulate in the fish liver (Chowdhury *et al.*, 2004). Copper-induced histological alterations are found in the gill, kidney hematopoietic tissue, mechanoreceptors, chemoreceptors, and other tissues (Sorensen, 1991). Morphological and histological alterations in liver of fishes exposed to copper have been documented (Varanka *et al.*, 2001). Higher doses of copper caused visible external lesions such as discoloration and necrosis on livers of *Cyprinus carpio*, *Carassius auratus* and *Corydoras paleatus* (Cavas *et al.*, 2005). (Arellano *et al.*, 1999) reported vacuolization of endothelial cells in fish liver by after copper exposure. Hepatocyte vacuolization, necrosis, shrinkage, nuclear pyknosis and increase of sinusoidal spaces were the distinct changes observed in the liver of copper-exposed fish (Figueiredo-Fernandes *et al.*, 2007). Exposure of Nile tilapia (*Oreochromis niloticus*) to sublethal levels of Cu has been shown to cause histopathological alterations in gills (edema; vasodilation of the lamellar vascular axis) and livers (vacuolation and necrosis) (Figueiredo-Fernandes *et al.*, 2007).

**Lead (Pb)**

Lead (Pb) is a persistent heavy metal which has been characterized as a priority hazardous substance (Sfakianakis *et al.*, 2015). Although Pb is a naturally occurring substance, its environmental concentrations are significantly increased by anthropogenic sources which include base metal mining, battery manufacturing, Pb-based paints and leaded gasoline (Monteiro *et al.*, 2011). Lead in water may come from industrial and smelter discharges; from the dissolution of old lead plumbing, lead containing pesticides, through precipitation, fallout of lead dust, street runoff, and municipal wastewater (Sepe *et al.*, 2003). Aquatic organisms bio accumulate Pb from water and diet, although there is evidence that Pb accumulation in fish, is most probably originated from contaminated water rather than diet (Cretì *et al.*, 2010). Lead deposits in various fish organs: liver, kidneys and spleen, but also digestive tract and gills (Jezierska B, Witeska M, 2006). Accumulation of lead in different fish species has been determined in several works (Castro-González *et al.*, 2008), leading to disorders in fish body. When *C. batrachus* exposed to 5 ppm of lead nitrate for 150 days, it exhibited marked inhibition of gonadal growth and showed decrease in cholesterol and lipid levels in brain, testis and ovary whereas the liver showed an elevation of both (Katti SR, Sathyanesan AG, 1983).

**Zinc (Zn)**

Zinc (Zn) is the second most abundant trace element after Fe and is an essential trace element and micronutrient in living organisms, found almost in every cell and being involved in nucleic acid synthesis and occurs in many enzymes (Sfakianakis *et al.*, 2015). Additionally, Zn is involved in more complicated functions, such as the immune system, neurotransmission and cell signaling (Hogstrand C, 2011). It may occur in water as a free cation as soluble zinc complexes, or can be

adsorbed on suspended matter. Zinc and its compounds are extensively used in commerce and in medicine. The common sources of it are galvanized ironwork, zinc chloride used in plumbing and paints containing zinc (Clarke *et al.*, 1981). Zinc wastes can have a direct toxicity to fish at increased waterborne levels (Niyogi S, Wood CM, 2006), and fisheries can be affected by either zinc alone or more often together with copper and other metals (Alabaster JS & Lloyd R, 1982).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.0 Experimental Design

The experiment was conducted in the wet laboratory of the Department of Fisheries and Aquaculture in the Federal University Oye-Ekiti, Ekiti State, Nigeria. The experiment was carried out in 10 plastic containers which served as the aquaria, each container was filled with 10litre of water and 10 juvenile *Clarias gariepinus* making it a total of 100 fish. The experiment was carried out in duplicates. The fish species *Clarias gariepinus* was exposed to four different concentration of Antifouling paint of 0.75ml/L, 1.00ml/L, 1.25ml/L and 1.50ml/L and a control which contained no paint. Water quality parameter was tested at 24hours interval and after 96hours the test organism was taken to the Federal Medical Center Ido, Ekiti State for the hematological determination and histopathological examination while the test organism, aquaria water and the treatment was taken to the Central Research Laboratory of Federal University of Technology Akure, Ondo State for heavy metal analysis.

## 3.1 Collection and acclimatization of fish

A total of 300 healthy live *Clarias gariepinus* juvenile of mean weight and mean total length 40g and 10.5cm respectively, was bought and transported from Afe Babalola University Farms (ABUAD FARM) in oxygenated plastic bags to the Fisheries Laboratory of the Federal University of Oye- Ekiti, Ekiti State. Acclimation of fish was done in fresh water by gradually changing the water in the cylindrical tanks with water holding capacity of 22litres from 100% holding water to 100% dilution water for over 2days. The fish were fed daily to satiation during the first two weeks of

acclimation, with a pelleted commercial feed (40% crude protein) in order to remove any problem that could arise as a result of starvation. (Ariyomo 2008).

### 3.2 Range Finding Test

One hundred (100) juveniles of *Clarias gariepinus* of mean weight and mean total length 5.51g and 8.83cm respectively were used for the range finding test. Mettler top precision loading balance was used to weigh the fish individually, followed by unbiased stocking of fish into transparent cylindrical plastic containers for two days in order to adapt to laboratory conditions. Feeding was discontinued during this period to reduce the production of waste in the transparent cylindrical containers thus minimizing the chances of ammonia production. The range finding was conducted under standard bioassay procedures (American Public Health Association, 1977). The range-finding test was carried out using fifteen transparent cylindrical plastic containers of 22litres capacity each was filled with 10litres of water prior to the introduction of antifouling paint. Four varying concentration used were 0.0030mg/l, 0.0050mg/l, 0.0070mg/l, 0.0090mg/l and 0.0110mg/l. The four different concentrations was carried out in duplicate; duplicate of the control was also be prepared (Ariyomo 2008).

### 3.3 Definitive Test

One hundred (100) juveniles of *Clarias gariepinus* of mean weight and mean total length 5.51g and 8.83cm respectively were used for the range finding test. Mettler top precision loading balance was used to weigh the fish individually, followed by unbiased stocking of fish into transparent cylindrical plastic containers for two days in order to adapt to laboratory conditions. Feeding was discontinued during this period to reduce the production of waste in the transparent cylindrical containers thus minimizing the chances of ammonia production. Definitive tests were

conducted under standard bioassay procedures (American Public Health Association, 1977). The following concentrations were used for the definitive test; control which contained no treatment, 0.75ml/L, 1.00ml/L, 1.25ml/L and 1.50ml/L from treatment 1-4 respectively.

## 3.4 DETERMINATION OF PHYSIOCHEMICAL PARAMETERS IN THE AQUARIA

The physiochemical parameters of the water in the aquaria will be examined at every 24hours for the 96hour experiment using standard methods.

### 3.4.1 Dissolved Oxygen (DO) Concentration

DO was determined using a dissolved oxygen meter (DO model 90.71). The probe was inserted into the sample bottles containing the different treatments. The unit of measurement is mg/l.

### 3.4.2 pH

pH was determined by using pH meter (model, METTLER TOLEDO 320). The probe was inserted into the sample bottles containing the different sample, and readings were taken and recorded daily.

### 3.4.3 Temperature

The temperature was measured and recorded using a digital thermometer at 24hour interval.

## 3.5 DETERMINATION OF LETHAL CONCENTRATION $LC_{50}$

This value is defined as the concentration at which 50% of a test organism is killed by the introduction of the toxicant. Data collected on mortality was subjected to Probit and Logit transformation method (Finney, 1982) and the $LC_{50}$ value was determined accordingly.

## 3.6 HISTOLOGICAL EXAMINATION OF SOME SELECTED ORGANS OF *Clarias gariepinus*

Histological evaluation of flesh, liver and gills were carried out by cutting the tissue of the fish and washing in 0.9% NaOH to remove the adherence of mucous and blood. It was kept on the blotting paper to drain the moisture. The tissue samples were processed for logical observation. The gill, liver, and flesh of the fish groups were fixed in physiological saline solution for 24 hrs. Using tetra hydrofuron as a dehydrating and clearing agent. The section of 6µ thickness was selected to observe the changes in the flesh, liver and the gills by adding hematoxylin and Eosin counter stain under light microscope fitted with a camera (Sonia Mumford., *et al* 2007).Photographs of the stained specimens were finally taken and interpreted accordingly.

## 3.7 HEMATOLOGICAL EVALUATION OF FISH SAMPLE

Three (3) fishes were selected at random and the blood sample was collected from the fish by severing the caudal peduncle and the blood was collected with the aid of 2.5ml heparinised syringes already treated with Ethylene diamine tetra acetic acid (EDTA) to prevent coagulation. Packed cell volume (PCV), red blood cells, white blood cells and differential counts were estimated using various methods described by (Svobodova *et al.*, 1991).

### 3.7.1 **Packed Cell Volume**

The haematocrit value expresses the corpuscular volume in relation to the total volume of blood. In ichthyohaematology, heparinized capillaries 7.5 cm long are exclusively used for the determination of the haematocrit value. The freshly collected non-stabilized or heparinized blood (which may be left to stand at a temperature of up to 4 °C for 4 hours after collection at the maximum) was sucked into the capillaries to about ⅔ of their height and the clean end was sealed

over a burner. Then the capillaries was put into the centrifuge (speed 14 000 r.p.m.) and are left to be centrifuged for 3 minutes. After centrifuging, the haematocrit percentage was directly read on the hematocrit meter which was a part of the hematocrit centrifuge set. The percent value obtained in this way was multiplied by coefficient 0.01 and the resultant value is the PCV in $1.1^{-1}$.

### 3.7.2 Red Blood Cell

The erythrocyte count was determined in heparinized blood diluted by the Hayem solution at a ratio of 1:200.The flask (bublet) method after Bürker was used for the dilution of the blood. The blood was diluted in special glass bublets. First, a special pipette was used to put an accurate amount of 4975 μl of Hayem solution (filtered before use) into the bublet; then 25 μl of heparinized blood was added, using a flushing micropipette. The micropipette was rinsed several times by repeatedly sucking the solution, the bublet was closed with a rubber stopper and its content was stirred by circling motion for 2 to 3 min. A dropper was used to fill Bürker's counting cell with the diluted blood. The red blood cells were counted in 20 rectangles, regularly distributed over the whole lattice of the counting cell. The counting was done at a 200-fold magnification.

The resultant counted amount of erythrocytes was then reduced 100 times and the resultant value is the number of erythrocytes in $T.1^{-1}$ (Tera = $10^{12}$) (Z. Svobodová, B. Vykusová 1991).

### 3.7.3 White Blood Cell

The leucocyte count was determined in heparinized blood, diluted with a solution after Procházka and Škrobák at a ratio of 1:200. The Procházka-Škrobák solution had the following composition; sodium chloride NaCl 3.88g, sodium sulphate $Na_2SO4$ 2.50g, sodium monohydrophosphate dodecahydrate Na2HPO4.12H2O 2.91g, potassium dihydrophosphate $KH_2PO_4$ 0.25 ml, formaldehyde 37% 7.50 ml, brilliant cresil blue 0.10g, distilled water at 1000 ml. Fresh

(newly prepared) solution was not used for the determination itself, the solution was left to stand for about 2 weeks before it was filtered and used. The flask method after Bürker was used for diluting the blood. Special glass flasks (bublets) or penicillin phials (volume about 15 to 25 ml) was used for the dilution, first, 4975 µl of the Procházka-Škrobák solution was put in the flasks or phial by means of a special pipette and then 25 µl of heparinized blood was added by means of a rinsing micropipette. The solution was then sucked repeatedly several times to rinse the micropipette, the flask was closed by means of a rubber stopper and its content was stirred by a cycling motion for 2–3 minutes. A dropper or a Pasteur pipette was then used to fill Bürker's counting cells with the diluted blood. The leucocytes were counted in 100 large squares. The counting was done at a 200-fold magnification. The total number of leucocytes, counted in the 100 large squares, was multiplied by 0.5 to give the leucocyte count, expressed in $G.l^{-1}$ (G-giga = $10^9$) Svobodová Z., Pravda D., Paláčková J. (1991).

### 3.7.4 Differential cell count

The differential cell count of neutrophil, eosinophil, basophil, lymphocyte and monocyte were determined according to Z. Svobodová, B. Vykusová 1991 method.

### 3.8 Heavy metal analyses

Heavy metals concentration was determined using AOAC (1990) method. Two grams (2 g) of each of the samples was heated in a muffle furnace at 600°C until it changed to ash. Thirty milliliters (30ml) of 0.1M $H_2SO_4$ was used to digests the ash, and the solution will be made up to 100ml with deionized water and then filtered. The concentrations of these heavy metals in the gill and liver of *Clarias gariepinus* was measured by Atomic Absorption Spectrophotometer AAS; model SSI UV 2101 at the Central Research Laboratory of the Federal University of Technology Akure, Ondo State, Nigeria. The instrument setting and operational conditions was done in accordance with the

manufacturers' specifications. Results were expressed in mg/kg dry weight for the body parts and mg/litre for the water samples.

### 3.9 Statistical Analysis

The parameters such as heavy metal concentration, hematological properties and $LC_{50}$ of *Clarias gariepinus* exposed to different concentration of antifouling paint was subjected to One-way analysis of variance using the Statistical Package for Social Science Version 2.0. Parameters where significant difference occurred were separated using the Duncan new multiple range test at $p< 0.05$. The $LC_{50}$ of *Clarias gariepinus* was determined using the Biotic Ligand model version 2.0.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Water quality parameters

The water quality parameters that was tested were temperature, pH and dissolved oxygen concentration. It was measured at 24hours interval during the 96hours period of the experiment and the results are shown in the table1 below.

### 4.2 Behavioral observation of African catfish exposed to antifouling paint

The mortality of *Clarias gariepinus* exposed to varying concentrations of Antifouling paint was observed in 1.25ml/L and 1.50ml/L at 72hours and 96hours. The mortality is recorded in table 2 below. On addition of antifouling paint, loss of reflex, air gulping, and barbels shortening was observed in higher concentrations of 1.00ml/L, 1.25ml/L and 1.50ml/L at 48hours, 72hours and 96hours consecutively. Rapid release of bubbles was also observed in higher concentrations of 1.00ml/L, 1.25ml/L and 1.50ml/L at 48hours and 96hours while death was observed in concentrations of 1.00ml/L, 1.25ml/L and 1.50ml/L at 72hours and 96hours.

### 4.3 Histological changes

The histological changes of the liver and gills of African catfish exposed to varying concentrations of Antifouling paint were examined. Examinations on the gills and livers showed varying degrees of damages. The gills of fish in the control showed remarkable morphology with no sign of necrosis and no signs of inflammation while the liver also showed normal morphology of hepatocyte with no signs of inflammation (Plate 1 & 6). In the concentration of 0.75ml/L, the gill was found to have a normal hepatocyte with mild inflammation while the gills had no necrosis and inflammatory infiltrates is unremarkable compared to the control (Plate 2 & 7).

There was mild steatosis, patchy lobular hepatocytes necrosis and mild to moderate inflammation with inflammatory cells nearly lymphocyte and no fibrosis in the liver of the fish in 10.0mg/L concentration while the gills showed no necrosis but there was significant lymphocytic inflammatory infiltrates (Plate 3 & 8). For higher concentrations of 1.25ml/L (Plate 4 &9), the liver was found to have moderate steatosis with multifocal hepatocytes necrosis and moderate inflammation but no fibrosis while the gills showed multifocal area of necrosis with moderate lymphocytic inflammatory infiltrates. The fish in the aquaria with the highest concentration of 1.50ml/L was found to have liver with diffuse lobular hepatocyte necrosis with severe inflammation but no fibrosis while the gills had severe necrosis with severe inflammation (Plate 5 & 10). The result obtained showed that Antifouling paint has direct effect on the tissue of the liver and gills of *Clarias gariepinus*.

### 4.4 Hematological examination

The concentrations of antifouling used were 0.75ml/L, 1.00ml/L, 1.25ml/l and 1.50ml/l the result obtained from the analysis showed that the values of the blood indices either increased or decreased with increasing concentrations. The results showed that parameters like Packed Cell Volume and Red Blood Cell count obtained after the 96hours reduced compared to the values of the control, it showed values which decreased with increasing concentrations of the antifouling paint with the highest values at control and the lowest at concentration of 1.50ml/L (Table 3). The reduction in the RBC may be due to the presence of stressors which manifest in form of a change in the environment resulting to haemagglutination due to impaired osmoregulation (Rottman *et al.*, 1992) or erythropoiesis in the organs responsible for the production of RBC. Also, the PCV value of the fish species was observed to reduce with increasing concentration of crude oil and exposure time of the fish species. This may be attributed to the changes in water

balance, which could cause a decrease in blood volume and an increase in the white blood cells resulting in reduced PCV (Cameron, 1970). White Blood Cell count increased with increasing concentration of antifouling paint, it had lowest values at control compared to higher concentrations of 1.00ml/L, 1.25ml/L and 1.50ml/L. The increase in WBC may be due to recruitment of more cells to combat the stressor (Ajani *et al.*, 2007). This increase may also be attributed to non- specific immune response to stress as a result of interaction of prolactin and cortisol hormones to restore ion balance in isosmotic salinity (Anyanwu *et al.*, 2007), and a stimulation of the immune system in response to toxicity of Antifouling paint. The hematological result showed that antifouling paint had significant effects on the Packed cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC) and the differential cell counts of the test organism; African Catfish (*Clarias gariepinus)* with control having the highest values of 13 ± 0.47, 1.10 ± 0.01, 1.67 ± 0.67 and 2.67± 0.33 in PCV, RBC, Neutrophil and Monocyte respectively. (Table 3)

### 4.5 Heavy metal analysis

The result observed from the laboratory analysis of the heavy metal content of the fish sample and water sample across concentration is showed in Table 4 below. Upon analysis, it was observed that the Antifouling Paint used had the heavy metals; Copper (Cu), Zinc (Zn), Lead (Pb) and Iron (Fe) in varying proportion. Consequently, after the 96hour period, it was observed that the heavy metal present in water sample and fish sample both increased with increase in concentration of Antifouling Paint. Concentrations with higher amount of Antifouling Paint had more deposits of Cu, Zn, Pb and Fe in the water and fish tissue. It was also observed in this study that there was increase in each heavy metal concentration with high concentration of copper in antifouling paint. Copper was found to cause immunosuppression of antibody producing cells in

rainbow trout when tested in vitro (Khangarot *et al.*, 1991), Defense against internal infections can be compromised by prolonged exposure to Copper (Malins *et al.*, 1988). The cause of mortality may be the toxicity of heavy metals in fish. Exposure of fish to Lead for up to 183 days was reported to produce a reduction in spleen size but an increase in leukocyte number and an especially large increase in the number of thrombocytes (Robohm, 1986) so this could be one of the reasons for increase in WBC value of *Clarias gariepinus* exposed to sublethal concentration of antifouling paint. In fish exposed to lead for at least 60 days, lymphoid tissue in head kidney was greatly reduced. Similar changes were reported in case of fish chronically exposed to Zinc (Rougier *et al.*, 1994).

**Table 1:** Physiochemical Parameters of Test Medium

| Variation | Parameters | Temperature ( °C ) | pH | Dissolved Oxygen (mg/L) |
|---|---|---|---|---|
| Hours | 24 Hour | $23.96 \pm 0.22^{a}$ | $7.87 \pm 0.01^{a}$ | $7.62. \pm 0.74^{b}$ |
| | 48 Hour | $26.84 \pm 0.22^{c}$ | $7.90 \pm 0.01^{b}$ | $7.68 \pm 0.74^{a}$ |
| | 72 Hour | $24.61 \pm 0.22^{b}$ | $7.85 \pm 0.01^{a}$ | $7.88 \pm 0.74^{a}$ |
| | 96 Hour | $26.75 \pm 0.22^{c}$ | $7.86 \pm 0.01^{a}$ | $7.94 \pm 0.74^{a}$ |
| Treatment | Control | $25.26 \pm 0.25^{a}$ | $7.88 \pm 0.01^{b}$ | $8.98 \pm 0.83^{d}$ |
| | 7.5mg/L | $25.39 \pm 0.25^{a}$ | $7.87 \pm 0.01^{ab}$ | $7.84 \pm 0.83^{b}$ |
| | 10.0mg/L | $25.19 \pm 0.25^{a}$ | $7.87 \pm 0.01^{b}$ | $7.03 \pm 0.83^{c}$ |
| | 12.5mg/L | $25.65 \pm 0.25^{ab}$ | $7.88 \pm 0.01^{b}$ | $7.48 \pm 0.83^{ab}$ |
| | 15.0mg/L | $26.20 \pm 0.25^{b}$ | $7.84 \pm 0.01^{a}$ | $6.98 \pm 0.83^{a}$ |

Mean ± S.E with different superscript across row is significantly different at $p < 0.05$

**Table 2**: Observations Recorded on the Behavior of *Clarias gariepinus*

| Observations | 24Hours | | | | | 48Hours | | | | | 72Hours | | | | | 96Hours | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.0 ml | 7.5 ml | 10.0 ml | 12.5 ml | 15 ml | 0.0 ml | 7.5 ml | 10. 0ml | 12.5 ml | 15.0 ml | 0.0 ml | 7.5 ml | 10. 0ml | 12.5 ml | 15.0 ml | 0.0 ml | 7.5 ml | 10.0 ml | 12.5 ml | 15.0 ml |
| Erratic Swimming | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Loss of reflex | - | - | - | - | - | - | - | + | + | + | - | - | + | + | + | - | - | + | + | + |
| Air gulping | - | - | - | - | - | - | - | + | + | + | - | - | - | + | + | - | - | + | + | + |
| Barbels shortening | - | - | - | - | - | - | - | + | + | + | - | - | + | + | + | - | - | + | + | + |
| Rapid release of bubbles | - | - | - | - | - | - | - | + | + | + | - | - | - | - | - | - | - | - | - | - |
| Death | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | - | - | + | + | + |

Keys: += present, - =not present

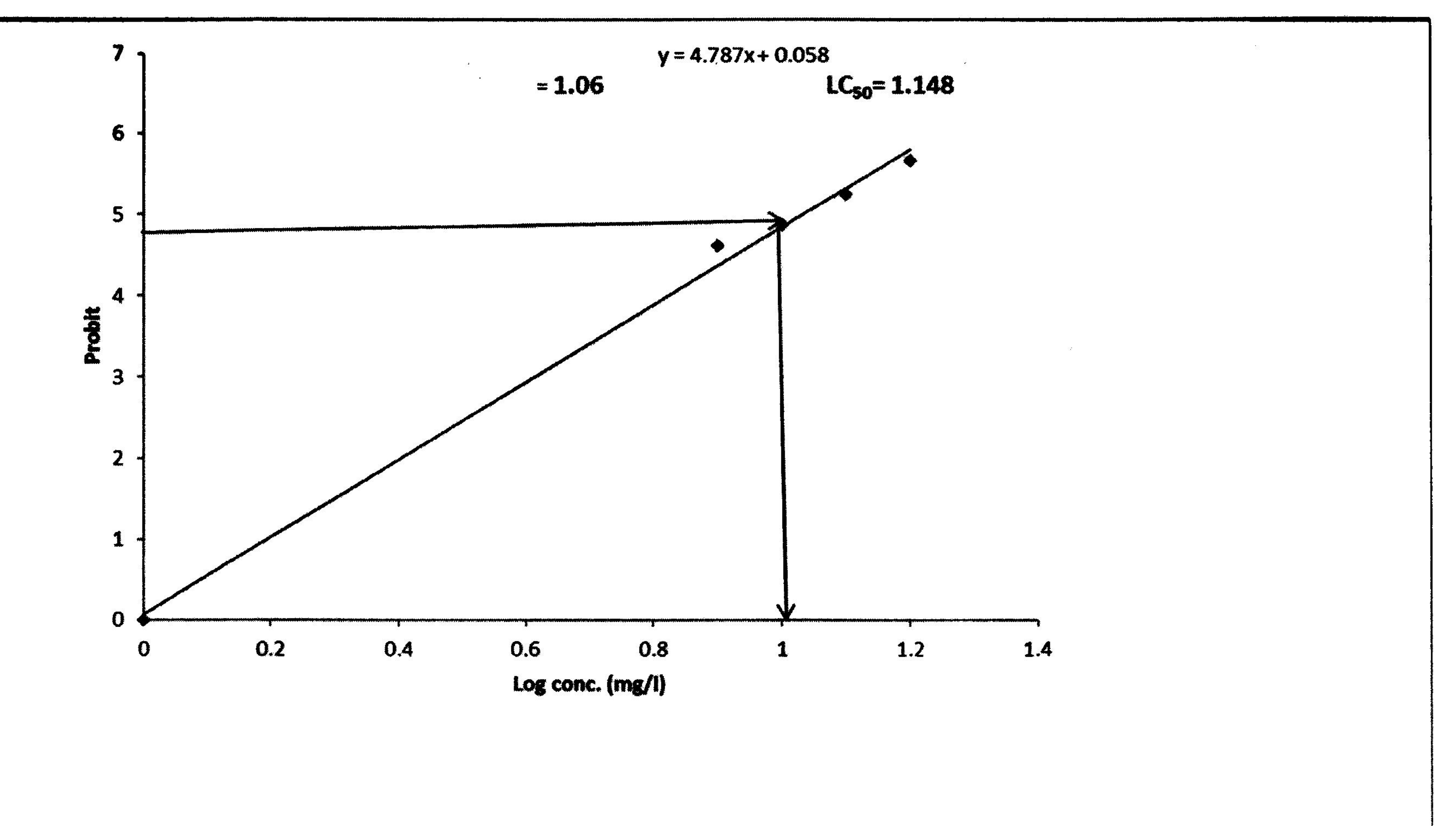



Figure 2: Mortality (96h – $LC_{50}$) of *Clarias gariepinus* in different concentrations of Antifouling Paint

**Table 3**: Hematological Parameter of African Catfish Exposed to Varying Concentration of Antifouling Paint

| | TREATMENT | | | | |
|---|---|---|---|---|---|
| **PARAMETERS** | **CONTROL** | **7.5mg/L** | **10.0mg/L** | **12.5mg/l** | **15.0mg/L** |
| **Pack Cell Volume (%)** | $13.7 \pm 0.47^{c}$ | $13.53 \pm 0.35^{c}$ | $10.57 \pm 0.23^{b}$ | $9.83 \pm 0.09^{b}$ | $8.67 \pm 0.24^{a}$ |
| **White Blood Cell ($*10^{9}$/L)** | $40.03 \pm 1.13^{a}$ | $54.17 \pm 0.67^{b}$ | $56.63 \pm 0.80^{b}$ | $71.23 \pm 1.48^{c}$ | $102.1 \pm 1.93^{d}$ |
| **Red Blood Cell ($*10^{12}$/L)** | $1.10 \pm 0.01^{d}$ | $1.03 \pm 0.07^{bc}$ | $0.95 \pm 0.03^{ab}$ | $0.90 \pm 0.01^{a}$ | $0.85 \pm 0.01^{a}$ |
| **Neutrophil (%)** | $2.67 \pm 0.67^{b}$ | $1.00 \pm 0.00^{ab}$ | $0.87 \pm 0.33^{ab}$ | $0.67 \pm 0.33^{ab}$ | $0.34 \pm 0.00^{a}$ |
| **Eosinophil (%)** | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ |
| **Basophil (%)** | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ |
| **Lymphocyte (%)** | $94.33 \pm 1.76^{a}$ | $97.00 \pm 0.56^{ab}$ | $97.33 \pm 0.88^{b}$ | $98.00 \pm 0.58^{b}$ | $98.40 \pm 1.15^{b}$ |
| **Monocyte (%)** | $2.87 \pm 0.33^{c}$ | $2.00 \pm 0.58^{bc}$ | $1.87 \pm 0.33^{a}$ | $1.47 \pm 0.33^{a}$ | $1.28 \pm 0.00^{ab}$ |

Mean ± S.E with different superscripts within column are significantly different at $p < 0.05$

**Table 4a**: Heavy Metal in Water Sample exposed to varying concentration of Antifouling Paint

| | | TREATMENTS | | | | National and International Standards | |
|---|---|---|---|---|---|---|---|
| | | WATER (ml/L) | | | | Water (ml/L) | |
| METALS | PAINT (ppm) | 0.75ml/L | 1.00ml/L | 1.25mg/L | 1.50ml/L | FDA | WHO |
| Cu | 5700 | $0.65 \pm 0.012^{a}$ | $0.85 \pm 0.046^{b}$ | $1.49 \pm 0.047^{c}$ | $1.95 \pm 0.059^{d}$ | 1.0 | 1-2 |
| Zn | 2300 | $11.9 \pm 6.17^{a}$ | $13.2 \pm 5.24^{a}$ | $15.4 \pm 2.60^{b}$ | $16.1 \pm 4.33^{c}$ | - | 15.0 |
| Pb | 163 | $0.03 \pm 0.002^{a}$ | $0.05 \pm 0.008^{a}$ | $0.08 \pm 0.012^{a}$ | $0.42 \pm 0.029^{a}$ | 0.005 | 0.01 |
| Fe | 560 | $0.11 \pm 0.004^{a}$ | $0.13 \pm 0.002^{a}$ | $0.24 \pm 0.049^{b}$ | $0.37 \pm 0.023^{c}$ | - | 0.3 |

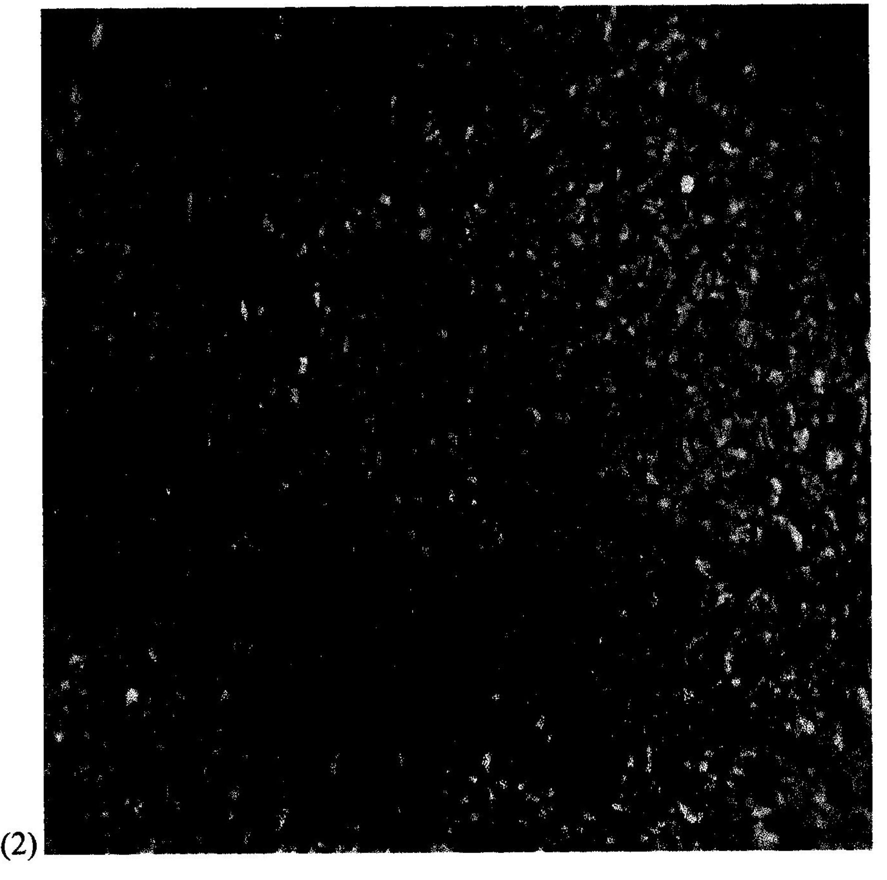
Mean ± S.E with different superscripts within column are significantly different at $p < 0.05$

| | TREATMENTS | | | | | National and International Standards | |
|---|---|---|---|---|---|---|---|
| | | FISH (mg/l) | | | | Fish (mg/l) | |
| METALS | PAINT (ppm) | 0.75ml/L | 1.00ml/L | 1.20ml/L | 1.50ml/L | EPA | WHO |
| Cu | 5700 | $0.39 \pm 0.04^{a}$ | $0.72 \pm 0.05^{b}$ | $1.11 \pm 0.14^{c}$ | $1.25 \pm 0.02^{c}$ | 1.0 | - |
| Zn | 2300 | $0.357 \pm 2.33^{a}$ | $0.523 \pm 6.23^{b}$ | $0.797 \pm 4.84^{c}$ | $0.853 \pm 2.60^{d}$ | 0.5 | - |
| Pb | 163 | $0.005 \pm 0.001^{a}$ | $0.008 \pm 0.001^{a}$ | $0.026 \pm 0.003^{b}$ | $0.069 \pm 0.005^{c}$ | 0.05 | 1.5 |
| Fe | 560 | $0.07 \pm 0.010^{a}$ | $0.08 \pm 0.005^{a}$ | $0.17 \pm 0.002^{b}$ | $0.29 \pm 0.012^{c}$ | 0.1 | - |

Table 4b: Heavy Metal in fish Sample exposed to varying concentration of Antifouling Paint

Mean ± S.E with different superscripts within column are significantly different at $p < 0.05$

**Histology of Liver and Gill of *C. gariepinus* exposed to varying concentrations of Antifouling paint**



(1) (2)

(3)

(4)

(5)

**PLATE 1**: **Liver of** ***Clarias gariepinus*** in the control without Antifouling Paint showing normal morphology of **hepatocyte with no inflammation.**

**PLATE 2**: Liver of *Clarias gariepinus* exposed to the 0.75ml/L Antifouling Paint showing normal hepatocyte with mild **inflammation.**

PLATE 3: Liver of *Clarias gariepinus* exposed to 1.00ml/L Antifouling Paint showing mild steatosis, patchy lobular and hepatocyte necrosis.

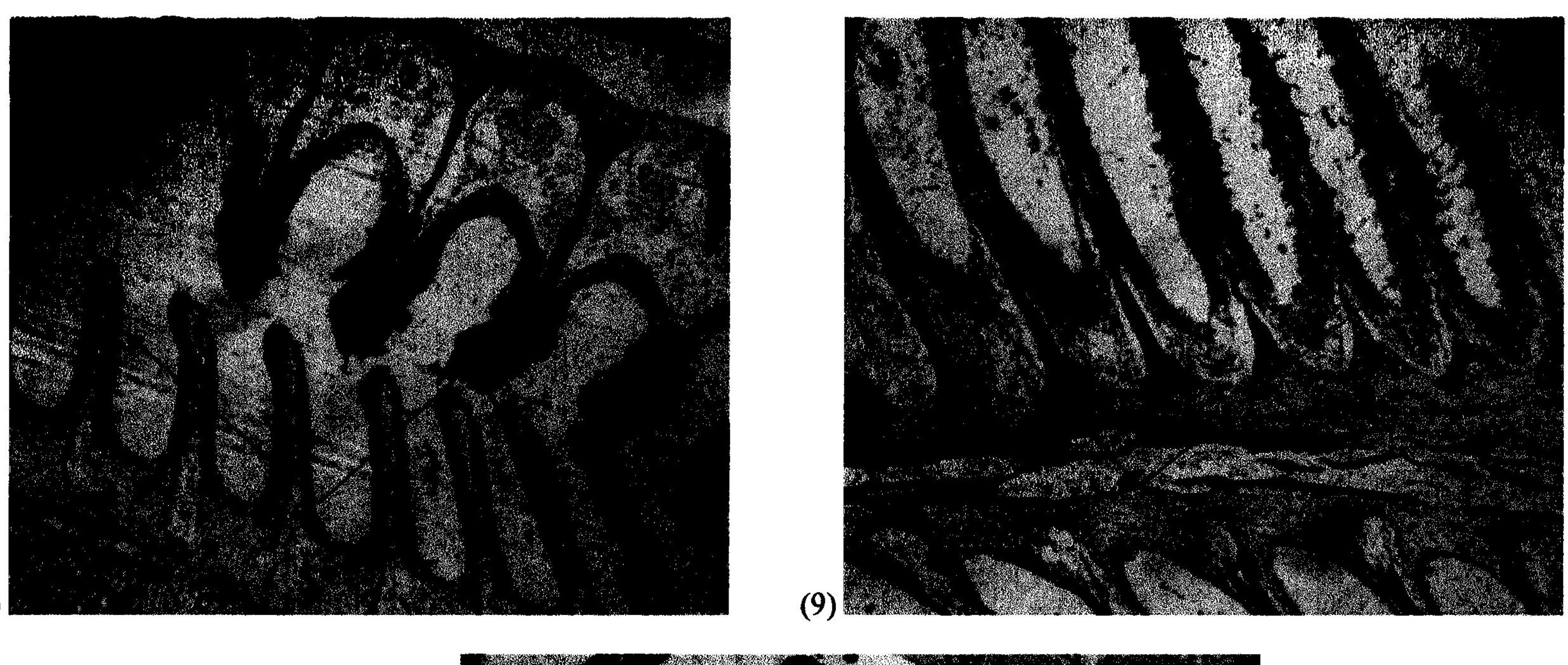
PLATE 4: Liver of Clarias gariepinus exposed to 1.25ml/L Antifouling Paint showing moderate steatosis with multifocal hepatocyte necrosis and inflammation but no fibrosis.

PLATE 5: Liver of *Clarias gariepinus* exposed to 1.50ml/L Antifouling Paint showing diffuse lobular hepatocytes necrosis with severe inflammation but no fibrosis.

(6)

(7)

(8) (9)



(10)

**PLATE 6: Gill of *Clarias gariepinus* in the control concentration without Antifouling Paint showing remarkable morphology with no sign of necrosis or inflammation.**

PLATE 7: Gill of *Clarias gariepinus* exposed to 0.75ml/L Antifouling Paint showing unremarkable morphology with no necrosis and inflammatory infiltrate is unremarkable compared to the control.

PLATE 8: Gill of *Clarias gariepinus* exposed to 1.00ml/L Antifouling Paint showing no sign of necrosis but with significant lymphocytic inflammatory infiltrates.

PLATE 9: Gill of *Clarias gariepinus* exposed to 1.25ml/L Antifouling Paint showing multifocal area of necrosis with moderate lymphocytic inflammatory infiltrates.

PLATE 10: Gill of *Clarias gariepinus* exposed to 15.0mg/L Antifouling Paint showing severe necrosis with severe inflammation.

# CHAPTER FIVE

## 5.0 DISCUSSION

*Clarias gariepinus* is one of the most cultivated freshwater fish worldwide. This study was designed to determine if antifouling paint can induce stress on *Clarias gariepinus* juvenile by affecting the hematology and histology of the fish.

Upon the addition of antifouling paint, behavioral changes were observed in the fish across the concentrations, some of these changes were loss of reflex, air gulping, rapid release of bubble and barbels shortening. After the introduction of the treatments to the water of the aquaria, the fish showed signs of stress which occurred due to the loss of dissolved oxygen as a result of Mechalin antifouling covering the top of the water. There was rapid vertical movement of the fish and rapid release of bubbles at the surface but this increased with higher concentration of AF compared to the control. At higher concentration of antifouling paint of 1.00ml/L, 1.25ml/L and 1.50ml/L during 48hours-96hours, the loss of reflex was observed in the fish, this was as a result of hyper activity and rapid opercula movement along and these correspond to the discovery of Van-Dyk *et al.* (2007) that found that the harmful effects of heavy metals pollution of fish depend on the duration of the exposure (chronic or acute) and the concentration level of the specific metal. Behavioral changes were not observed at low effluent concentrations, this shows that fishes tolerated low concentrations of the pollutants thus the observed reduced mortality. Consequently, it was observed in this study that the abnormal behavior and mortality rate of the test organisms increased with increase in the concentrations of pollutant. This corresponds to the findings of Shobha K, *et al.*; (2007) who found that the behavior and mortality rate of *Catla catla* during experimentation was found to depend on both the duration of exposure

and concentration of the toxicant. This also corresponds with the findings of Oyedapo and Akinduyite, (2011) that when the concentration of the toxic substance is higher than what the homeostasis of the fish can control, it result in death and cause damages in the fish opercula and may also cause physical damages to fish particularly on the skin, liver and gill surface.

The result obtained from the analysis of the physiochemical parameters of both the control and the other concentrations used during the experiment are shown in Table 1 above. The result obtained revealed that there is significant difference between the control and varying concentrations. No change was recorded in the temperature and pH throughout the course of the experiment. The implication of the pH result obtained from this study is that there is no risk since it falls within the Federal Environmental Protection Agency limit. For best fish production, Ayodele and Ajani (1999) recommended a pH of range of 6.5 - 9 while Kunle (2000) recommended a pH range of 6.5 and 8.5. Mortality of fish could have being as a result of reduction in dissolved oxygen concentrations observed in the higher concentrations. This conforms with the report of Enujiugha and Nwanna (2004) who reported that reduction of oxygen levels can severely affect fish life causing stress and eventually causing mortality.

Microscopic examination of target tissues is an important end point in the evaluation of toxic potential and risk assessment of chemicals in the environment (Velma and Tchounwou, 2010). Gills are directly affected by contaminants owing to their direct and continuous contact with the external medium and their functions in respiratory gas exchange, osmoregulation, excretion of nitrogenous waste, and acid-base regulation (Bhagwant and Elahee 2002). The result of this study demonstrates that the liver of control fish exhibits a normal hepatocyte and there were no pathological abnormalities compared to the concentration of 0.75ml/L, 1.00ml/L, 1.25ml/L, 1.50ml/L (Plate 1-5) where steatosis (fatty liver disease), patchy lobular, hepatocyte

necrosis and inflammation were observed. This agrees with the result of Loganathan *et al.* (2006) who reported severe necrosis, inflammation and degeneration of hepatocytes in the liver tissue of *Labeo rohita* exposed to zinc. It showed that with increase in concentration of antifouling paint the resultant effects become more severe on the liver. Hepatocyte necrosis of the liver tissues observed in this study was because of the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification by the liver. The inability of fish to regenerate new liver cells may also have led to necrosis (El-Naggar *et al.*, 2009). Alterations in liver hepatocytes which includes the formation of vacuoles in the hepatocytes and patchy lobular associated with stress have been well studied and reported, this is as a result of histopathological lesion on liver tissue. (Metelev *et al.,* 1971).

The examination of the gills showed that the control fish did not undergo any significant changes in morphology, and no signs of necrosis or inflammation compared to the fish exposed to antifouling paint which showed degeneration of the morphology of the gill, significant lymphocytic inflammatory infiltrates, necrosis and inflammation, this conforms with the findings of Winkaler *et al.* (2001) and Tkatcheva *et al.* (2004) who reported that gills may change morphologically in order to maintain physiological functions under effects of pollution. When the gills are damaged, hypoxic condition occurs due to alteration in the gas exchange mechanisms (Das and Mukherjee, 2003).

Hematological indices are of different sensitivity to various environmental factors and chemicals (Vosyliene, 1999). According to Van Vuren, (1986) Studies have shown that when the water quality is affected by toxicants, any physiological change will be reflected in the values of one or more of the hematologic parameters. Thus water quality is one of the major factors responsible for individual variations in fish hematology since they are sensitive to slight

fluctuations that may occur within their internal milieu (Fernandez and Mazon, 2003). In this study, it was observed that White Blood Cell increased with increase in concentration of antifouling paint, this could be due to the attempt of the fish to fight against the pollutants which may lead to production of more WBC to improve the health status of the fish thus, this is similar to those reported in *C. gariepinus* exposed to petroleum oil and *Parkia bioghobossa* pods extracts (Adewoye 2010). The increase in WBC with increase in concentration observed in this study is similar with findings of (Nath and Banerjee, 1995; Mazon *et al.,* 2002) who reported significantly higher WBC in fish exposed to increased copper concentration. Mishra and Srivastava (1980) also reported an increase in leucocytes count when they exposed fishes to heavy metals.

Furthermore, upon exposure of *C. gariepinus* to antifouling paint, RBCs count showed decreasing trend and lowest value 0.85 ± 0.01 was observed at 15mg/L concentration. This conforms with the result of the study conducted by Holcombe *et al.* (1976) who found that there was decline in RBC values of brook trout (*Salvalinus fontinalis)* on exposure to long term effects of lead. It was suggested that the reduction in the RBCs counts during treatment, may be due to the development of hypoxia that led to either increase in the destruction of RBS or decrease in the genesis of RBCs due to non-availability of Hb content in cellular medium (Akinrotimi *et al.*, 2012; Vasantharaja *et al.*, 2012). The lymphocyte was observed to increase with increase in concentration of antifouling paint, this is corroborated by Srivastava and Narain (1982) who also reported an increase in the number of lymphocytes in *Heteropneustes fossillis* treated with endrin and nuvacron. Similarly, lymphocytosis was reported in *Ictalurus punctatus* subjected to hypoxia (Grizzle and Rogers, 1976; Scoot and Rogers, 1981). In fish, lymphocytes participate in inflammatory processes (Martins, 2000), although the function of these cells remains unclear

(Tavares-Dias; Moraes, 2004). It is possible that they are recruited to the focus on the lysate by the defense mechanisms, which might explain the high numbers of these cells in the blood circulation of the infected *C. gariepinus.* According to Ayandiran *et al.* (2010) and Gabriel *et al.* (2011), normal PCV values usually fall within the range of 20-35% and are rarely greater than 50% for fish. However, from the result of this study which showed PCV decrease to as low as 8.67% for the highest concentration of 1.50ml/L this could be because transport of metals in fish occurs through the blood where the ions are usually bound to proteins (Ayandiran *et al.*, 2009); and pollutants generally produce relatively rapid changes in blood characteristics of fish (Johansen *et al.*,1994; Moussa *et al.*,1994; Ezzat *et al.*, 1998; Rizkalla *et al.*, 1999).

The heavy metal observed in this study showed that Cu, Zn, Pb, Fe concentration in the water sample was more than that of the fish sample. This shows that fish takes up and bio-accumulates heavy metals in various quantities depending on their concentrations in the water. This agrees with the results of Oluyemi *et al* (2008) and Kemdrin (1979) who reported on the level of heavy metals in aquatic organism from different water bodies. Their results showed that aquatic animals (fish inclusive) bio-accumulate heavy metals in a considerable amount, and because these metals are not bio-degradable, they tend to stay in the fish tissues for a very long time which upon consumption of these fishes, the heavy metals get transferred to man, leading to heavy metal poisoning especially if present in higher concentrations.

## 5.1 CONCLUSION

Findings from this study indicated that exposure of *Clarias gariepinus* to antifouling paint induced stress and was reflected in a direct physiological effect resulting in significant effect on the hematological indices and histology of *Clarias gariepinus.* The gradual changes at lower concentration of antifouling paint in fish behavior reflected a transient stress induced osmotic imbalance. However, the serious changes observed showed that stress reduced the immune potential of fish. This reduced immunological status which persisted resulted in mortality especially at higher concentrations. Thus, it seems that even an incidental toxic stress may result in a considerable increase in susceptibility of fish to infections.

The changes in the hematological parameters indicate that they can be used as indicators of antifouling paint related stress in fish on exposure to elevated levels in the water. Exposure of *C. gariepinus* to higher concentrations of antifouling paint demonstrated a toxic poisoning. The report in the study may also infer that higher mortality is expected under a static bioassay method.

In addition, the results observed reinforce the high potential of histopathological lesions to reveal the effects of chronic and acute exposure of fish to pollutants, thus increasing the credibility of the diagnosis in impacted studies of water quality of aquatic ecosystems. Contamination of aquatic environment by heavy metals whether as a consequence of acute or chronic events constitutes additional source of stress for aquatic organisms. Sub-lethal concentrations of toxicants in the aquatic environment will not necessarily result in outright mortality of aquatic organisms. Toxicants and pollutants can result in several physiological dysfunctions in fish which could induce changes in blood parameters and target organs (Adakole

*et al.,* 2012). Hence, good knowledge of fish response to various stressors will be immensely helpful in improving production of fish and in providing information on ways of effectively controlling and monitoring stress in aquaculture.

## 5.2 RECOMMENDATIONS

Since it has been shown by this study that fish is affected by pollutions especially by antifouling paint, some steps that can be taken to protect the fish, its ecosystem and the humans who consume such fish are;

There is need for sensitization and massive awareness drive to activate the consciousness of the fishers, ship building companies, boat painters and paint manufacturing companies to the effect of their activities on the water body and the fishery resources in general.

There is need for investigations of water bodies around shipping docks and harbors as they are the areas mostly affected because the surrounding waters is more shallow and as such there will be higher concentration of antifouling paint leaching in such area due to either docking or mooring of vessels near such area or release of effluent in such areas by boat painters.

Fisheries extension officers should sensitize the aquaculturist on the need to desist from painting their fishing gear with antifouling paint as it can introduce heavy metals and biocides into the water which can be taken up by the fish.

The use of copper based antifouling paint should be favored over other types like biocide and organotin based paints because the use of copper-based antifouling paints provide clear advantages in the protection of the environment. Aside from the advantages of copper antifouling over organotin antifouling on marine life, most of the dissolved copper is complex

with organic and particulate material in marine and freshwater environments. This reduces or completely removes its ability to be taken up by organisms (i.e. its bioavailability) and therefore toxicity to marine organisms is unlikely.

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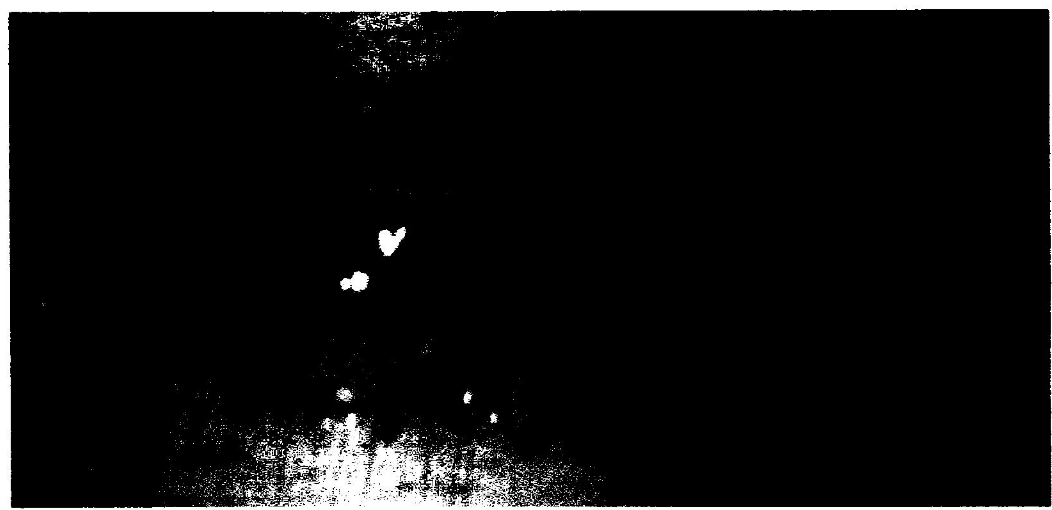
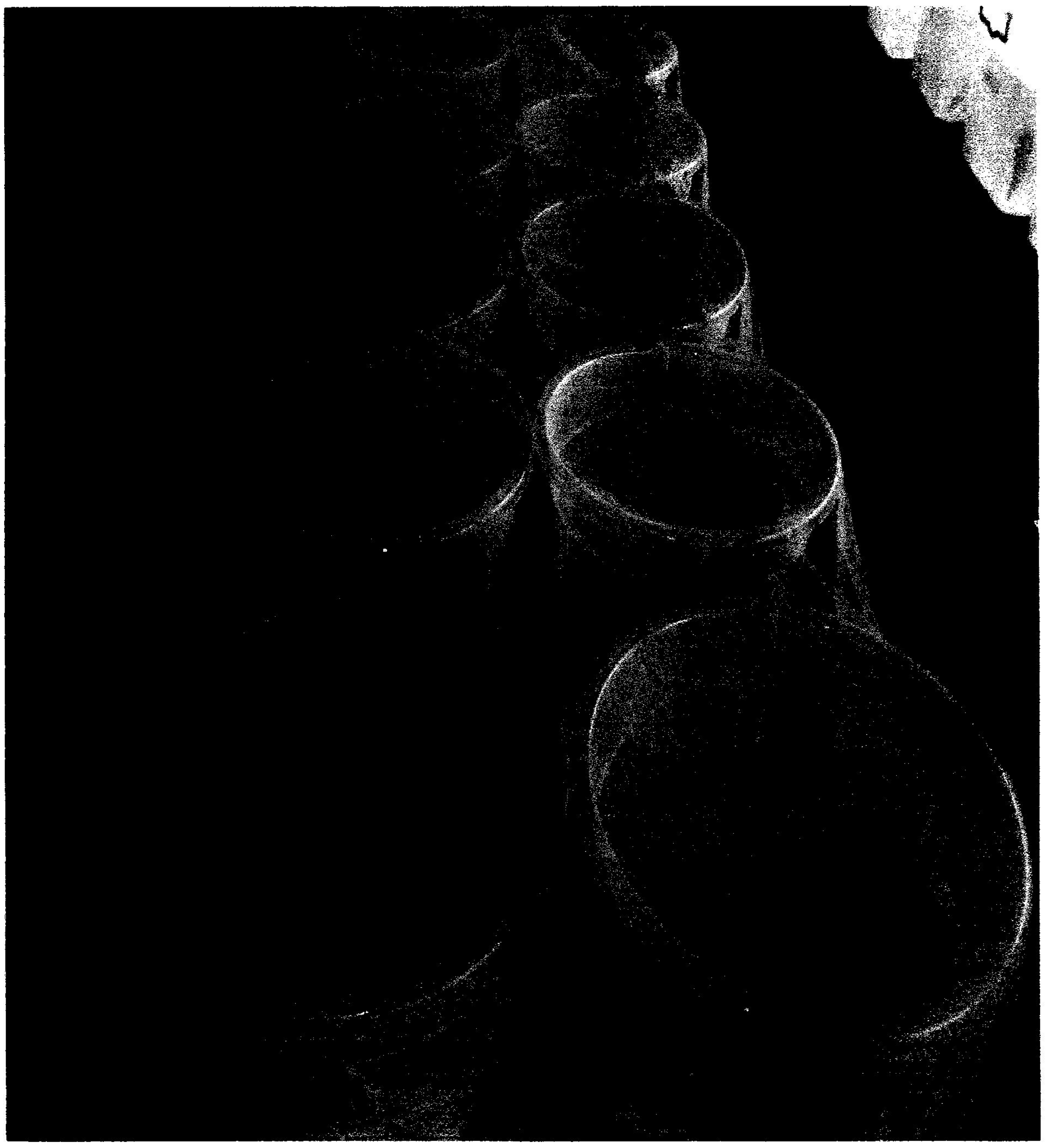
# APPENDICES

Mechalin Paint used as Antifouling Paint

Picture of *C. gariepinus*



Experimental setup



**Descriptive Statistics of physiochemical parameters of water of C. gariepinus exposed to varying concentrations of Antifouling paint**

| | hours | treatment | Mean | Std. Deviation | N |
|---|---|---|---|---|---|
| Do | 24hrs | control | 7.9767 | .64291 | 3 |
| | | 7.5mg/l | 7.5167 | .17243 | 3 |
| | | 10.0mg/l | 6.7200 | .23580 | 3 |
| | | 12.5mg/l | 6.2167 | .21825 | 3 |
| | | 15.0mg/l | 5.6100 | .07211 | 3 |
| | | Total | 6.8080 | .92909 | 15 |
| | 48hrs | control | 7.8300 | .42332 | 3 |
| | | 7.5mg/l | 6.5833 | .37166 | 3 |
| | | 10.0mg/l | 5.8567 | .23438 | 3 |
| | | 12.5mg/l | 5.5067 | .16442 | 3 |
| | | 15.0mg/l | 5.1100 | .10536 | 3 |
| | | Total | 6.1773 | 1.02067 | 15 |
| | 72hrs | control | 7.7967 | .12055 | 3 |
| | | 7.5mg/l | 6.1100 | .20000 | 3 |
| | | 10.0mg/l | 5.0667 | .29771 | 3 |
| | | 12.5mg/l | 4.4033 | .24379 | 3 |
| | | 15.0mg/l | 3.9033 | .22008 | 3 |
| | | Total | 5.4560 | 1.44501 | 15 |
| | 96hrs | control | 7.7300 | .25534 | 3 |
| | | 7.5mg/l | 6.6267 | .42829 | 3 |
| | | 10.0mg/l | 5.6633 | .47721 | 3 |

| | | | | | |
|---|---|---|---|---|---|
| | | 12.5mg/l | 4.7033 | .41741 | 3 |
| | | 15.0mg/l | 3.0300 | .13115 | 3 |
| | | Total | 5.5507 | 1.69686 | 15 |
| | Total | control | 7.8333 | .36210 | 12 |
| | | 7.5mg/l | 6.7092 | .59419 | 12 |
| | | 10.0mg/l | 5.8267 | .67847 | 12 |
| | | 12.5mg/l | 5.2075 | .77712 | 12 |
| | | 15.0mg/l | 4.4133 | 1.06332 | 12 |
| | | Total | 5.9980 | 1.38970 | 60 |
| Ph | 24hrs | control | 7.9067 | .03055 | 3 |
| | | 7.5mg/l | 7.8667 | .00577 | 3 |
| | | 10.0mg/l | 7.8633 | .01528 | 3 |
| | | 12.5mg/l | 7.8733 | .02082 | 3 |
| | | 15.0mg/l | 7.8333 | .01155 | 3 |
| | | Total | 7.8687 | .02900 | 15 |
| | 48hrs | control | 7.8733 | .01528 | 3 |
| | | 7.5mg/l | 7.8800 | .02000 | 3 |
| | | 10.0mg/l | 7.9333 | .03055 | 3 |
| | | 12.5mg/l | 7.9233 | .04726 | 3 |
| | | 15.0mg/l | 7.8967 | .02517 | 3 |
| | | Total | 7.9013 | .03502 | 15 |
| | 72hrs | control | 7.8867 | .00577 | 3 |

| | | | | | |
|---|---|---|---|---|---|
| | | 7.5mg/l | 7.8500 | .01000 | 3 |
| | | 10.0mg/l | 7.8000 | .03606 | 3 |
| | | 12.5mg/l | 7.8633 | .01528 | 3 |
| | | 15.0mg/l | 7.8433 | .00577 | 3 |
| | | Total | 7.8487 | .03335 | 15 |
| | 96hrs | control | 7.8700 | .01732 | 3 |
| | | 7.5mg/l | 7.8767 | .00577 | 3 |
| | | 10.0mg/l | 7.8967 | .04041 | 3 |
| | | 12.5mg/l | 7.8500 | .02646 | 3 |
| | | 15.0mg/l | 7.7933 | .02082 | 3 |
| | | Total | 7.8573 | .04217 | 15 |
| | Total | control | 7.8842 | .02234 | 12 |
| | | 7.5mg/l | 7.8683 | .01586 | 12 |
| | | 10.0mg/l | 7.8733 | .05805 | 12 |
| | | 12.5mg/l | 7.8775 | .03864 | 12 |
| | | 15.0mg/l | 7.8417 | .04130 | 12 |
| | | Total | 7.8690 | .03977 | 60 |
| temperature | 24hrs | control | 22.0000 | .00000 | 3 |
| | | 7.5mg/l | 23.3667 | .51316 | 3 |
| | | 10.0mg/l | 23.5667 | 1.02144 | 3 |
| | | 12.5mg/l | 24.2667 | .30551 | 3 |
| | | 15.0mg/l | 26.6000 | .55678 | 3 |

| | | | | |
|---|---|---|---|---|
| | **Total** | 23.9600 | 1.64003 | 15 |
| **48hrs** | **control** | 27.6000 | .26458 | 3 |
| | 7.5mg/l | 26.6333 | .15275 | 3 |
| | 10.0mg/l | 26.0667 | .23094 | 3 |
| | 12.5mg/l | 26.9333 | .15275 | 3 |
| | 15.0mg/l | 26.9667 | .05774 | 3 |
| | Total | 26.8400 | .53958 | 15 |
| 72hrs | control | 25.2667 | .35119 | 3 |
| | 7.5mg/l | 24.7667 | .30551 | 3 |
| | 10.0mg/l | 24.4000 | .17321 | 3 |
| | 12.5mg/l | 24.4000 | .20000 | 3 |
| | 15.0mg/l | 24.2000 | .10000 | 3 |
| | Total | 24.6067 | .44153 | 15 |
| 96hrs | control | 26.1667 | .15275 | 3 |
| | 7.5mg/l | 26.8000 | .00000 | 3 |
| | 10.0mg/l | 26.7333 | .20817 | 3 |
| | 12.5mg/l | 27.0000 | .10000 | 3 |
| | 15.0mg/l | 27.0333 | .15275 | 3 |
| | Total | 26.7467 | .34407 | 15 |
| Total | control | 25.2583 | 2.15763 | 12 |
| | 7.5mg/l | 25.3917 | 1.50179 | 12 |
| | 10.0mg/l | 25.1917 | 1.40030 | 12 |

| | 12.5mg/l | 25.6500 | 1.38728 | 12 |
|---|---|---|---|---|
| | 15.0mg/l | 26.2000 | 1.24389 | 12 |
| | Total | 25.5383 | 1.56130 | 60 |

**Estimated Marginal Means**

**1. hours * treatment**

| Dependent Variable | hours | treatment | Mean | Std. Error | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Do | 24hrs | control | 7.977 | .177 | 7.619 | 8.334 |
| | | 7.5mg/l | 7.517 | .177 | 7.159 | 7.874 |
| | | 10.0mg/l | 6.720 | .177 | 6.362 | 7.078 |
| | | 12.5mg/l | 6.217 | .177 | 5.859 | 6.574 |
| | | 15.0mg/l | 5.610 | .177 | 5.252 | 5.968 |
| | 48hrs | control | 7.830 | .177 | 7.472 | 8.188 |
| | | 7.5mg/l | 6.583 | .177 | 6.226 | 6.941 |
| | | 10.0mg/l | 5.857 | .177 | 5.499 | 6.214 |
| | | 12.5mg/l | 5.507 | .177 | 5.149 | 5.864 |
| | | 15.0mg/l | 5.110 | .177 | 4.752 | 5.468 |
| | 72hrs | control | 7.797 | .177 | 7.439 | 8.154 |
| | | 7.5mg/l | 6.110 | .177 | 5.752 | 6.468 |
| | | 10.0mg/l | 5.067 | .177 | 4.709 | 5.424 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 12.5mg/l | 4.403 | .177 | 4.046 | 4.761 |
| | | 15.0mg/l | 3.903 | .177 | 3.546 | 4.261 |
| | 96hrs | control | 7.730 | .177 | 7.372 | 8.088 |
| | | 7.5mg/l | 6.627 | .177 | 6.269 | 6.984 |
| | | 10.0mg/l | 5.663 | .177 | 5.306 | 6.021 |
| | | 12.5mg/l | 4.703 | .177 | 4.346 | 5.061 |
| | | 15.0mg/l | 3.030 | .177 | 2.672 | 3.388 |
| ph | 24hrs | control | 7.907 | .014 | 7.879 | 7.934 |
| | | 7.5mg/l | 7.867 | .014 | 7.839 | 7.894 |
| | | 10.0mg/l | 7.863 | .014 | 7.836 | 7.891 |
| | | 12.5mg/l | 7.873 | .014 | 7.846 | 7.901 |
| | | 15.0mg/l | 7.833 | .014 | 7.806 | 7.861 |
| | 48hrs | control | 7.873 | .014 | 7.846 | 7.901 |
| | | 7.5mg/l | 7.880 | .014 | 7.853 | 7.907 |
| | | 10.0mg/l | 7.933 | .014 | 7.906 | 7.961 |
| | | 12.5mg/l | 7.923 | .014 | 7.896 | 7.951 |
| | | 15.0mg/l | 7.897 | .014 | 7.869 | 7.924 |
| | 72hrs | control | 7.887 | .014 | 7.859 | 7.914 |
| | | 7.5mg/l | 7.850 | .014 | 7.823 | 7.877 |
| | | 10.0mg/l | 7.800 | .014 | 7.773 | 7.827 |
| | | 12.5mg/l | 7.863 | .014 | 7.836 | 7.891 |
| | | 15.0mg/l | 7.843 | .014 | 7.816 | 7.871 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | **96hrs** | control | 7.870 | .014 | 7.843 | 7.897 |
| | | 7.5mg/l | 7.877 | .014 | 7.849 | 7.904 |
| | | 10.0mg/l | 7.897 | .014 | 7.869 | 7.924 |
| | | 12.5mg/l | 7.850 | .014 | 7.823 | 7.877 |
| | | 15.0mg/l | 7.793 | .014 | 7.766 | 7.821 |
| temperature | 24hrs | control | 22.000 | .195 | 21.606 | 22.394 |
| | | 7.5mg/l | 23.367 | .195 | 22.973 | 23.761 |
| | | 10.0mg/l | 23.567 | .195 | 23.173 | 23.961 |
| | | 12.5mg/l | 24.267 | .195 | 23.873 | 24.661 |
| | | 15.0mg/l | 26.600 | .195 | 26.206 | 26.994 |
| | 48hrs | control | 27.600 | .195 | 27.206 | 27.994 |
| | | 7.5mg/l | 26.633 | .195 | 26.239 | 27.027 |
| | | 10.0mg/l | 26.067 | .195 | 25.673 | 26.461 |
| | | 12.5mg/l | 26.933 | .195 | 26.539 | 27.327 |
| | | 15.0mg/l | 26.967 | .195 | 26.573 | 27.361 |
| | 72hrs | control | 25.267 | .195 | 24.873 | 25.661 |
| | | 7.5mg/l | 24.767 | .195 | 24.373 | 25.161 |
| | | 10.0mg/l | 24.400 | .195 | 24.006 | 24.794 |
| | | 12.5mg/l | 24.400 | .195 | 24.006 | 24.794 |
| | | 15.0mg/l | 24.200 | .195 | 23.806 | 24.594 |
| | 96hrs | control | 26.167 | .195 | 25.773 | 26.561 |
| | | 7.5mg/l | 26.800 | .195 | 26.406 | 27.194 |

| | | | | | |
|---|---|---|---|---|---|
| | 10.0mg/l | 26.733 | .195 | 26.339 | 27.127 |
| | 12.5mg/l | 27.000 | .195 | 26.606 | 27.394 |
| | 15.0mg/l | 27.033 | .195 | 26.639 | 27.427 |

**2. hours**

| Dependent Variable | hours | Mean | Std. Error | 95% Confidence Interval | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| Do | 24hrs | 6.808 | .079 | 6.648 | 6.968 |
| | 48hrs | 6.177 | .079 | 6.017 | 6.337 |
| | 72hrs | 5.456 | .079 | 5.296 | 5.616 |
| | 96hrs | 5.551 | .079 | 5.391 | 5.711 |
| ph | 24hrs | 7.869 | .006 | 7.856 | 7.881 |
| | 48hrs | 7.901 | .006 | 7.889 | 7.914 |
| | 72hrs | 7.849 | .006 | 7.836 | 7.861 |
| | 96hrs | 7.857 | .006 | 7.845 | 7.870 |
| temperature | 24hrs | 23.960 | .087 | 23.784 | 24.136 |
| | 48hrs | 26.840 | .087 | 26.664 | 27.016 |
| | 72hrs | 24.607 | .087 | 24.430 | 24.783 |
| | 96hrs | 26.747 | .087 | 26.570 | 26.923 |

**4. treatment**

| Dependent Variable | treatment | Mean | Std. Error | 95% Confidence Interval | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |

| | | | | | |
|---|---|---|---|---|---|
| Do | control | 7.833 | .089 | 7.654 | 8.012 |
| | 7.5mg/l | 6.709 | .089 | 6.530 | 6.888 |
| | 10.0mg/l | 5.827 | .089 | 5.648 | 6.006 |
| | 12.5mg/l | 5.208 | .089 | 5.029 | 5.386 |
| | 15.0mg/l | 4.413 | .089 | 4.234 | 4.592 |
| ph | control | 7.884 | .007 | 7.870 | 7.898 |
| | 7.5mg/l | 7.868 | .007 | 7.855 | 7.882 |
| | 10.0mg/l | 7.873 | .007 | 7.860 | 7.887 |
| | 12.5mg/l | 7.877 | .007 | 7.864 | 7.891 |
| | 15.0mg/l | 7.842 | .007 | 7.828 | 7.855 |
| temperature | control | 25.258 | .097 | 25.061 | 25.455 |
| | 7.5mg/l | 25.392 | .097 | 25.195 | 25.589 |
| | 10.0mg/l | 25.192 | .097 | 24.995 | 25.389 |
| | 12.5mg/l | 25.650 | .097 | 25.453 | 25.847 |
| | 15.0mg/l | 26.200 | .097 | 26.003 | 26.397 |

**Post Hoc Tests**

**Hours**

**Homogeneous Subsets**

**Do**

Duncan

| hours | N | Subset 1 | 2 | 3 |
|---|---|---|---|---|
| 72hrs | 15 | 5.4560 | | |
| 96hrs | 15 | 5.5507 | | |
| 48hrs | 15 | | 6.1773 | |
| 24hrs | 15 | | | 6.8080 |
| Sig. | | .403 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .094.

**ph**

Duncan

| hours | N | Subset 1 | 2 | 3 |
|---|---|---|---|---|
| 72hrs | 15 | 7.8487 | | |
| 96hrs | 15 | 7.8573 | 7.8573 | |
| 24hrs | 15 | | 7.8687 | |
| 48hrs | 15 | | | 7.9013 |
| Sig. | | .318 | .193 | 1.000 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .001.

**Temperature**

**Duncan**

| hours | N | Subset | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 24hrs | 15 | 23.9600 | | |
| 72hrs | 15 | | 24.6067 | |
| 96hrs | 15 | | | 26.7467 |
| 48hrs | 15 | | | 26.8400 |
| Sig. | | 1.000 | 1.000 | .453 |

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .114.

**treatment**

**Homogeneous Subsets**

**Do**

Duncan

| treatment | N | Subset | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 15.0mg/l | 12 | 4.4133 | | | | |
| 12.5mg/l | 12 | | 5.2075 | | | |
| 10.0mg/l | 12 | | | 5.8267 | | |
| 7.5mg/l | 12 | | | | 6.7092 | |
| control | 12 | | | | | 7.8333 |
| Sig. | | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .094.

**ph**

**Duncan**

| treatment | N | Subset |
|---|---|---|

| | | 1 | 2 |
|---|---|---|---|
| **15.0mg/l** | **12** | **7.8417** | |
| **7.5mg/l** | **12** | | 7.8683 |
| **10.0mg/l** | **12** | | 7.8733 |
| **12.5mg/l** | **12** | | 7.8775 |
| **control** | 12 | | 7.8842 |
| Sig. | | 1.000 | .139 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .001.

**temperature**

Duncan

| treatment | N | Subset | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 10.0mg/l | 12 | 25.1917 | | |
| control | 12 | 25.2583 | | |
| 7.5mg/l | 12 | 25.3917 | 25.3917 | |
| 12.5mg/l | 12 | | 25.6500 | |
| 15.0mg/l | 12 | | | 26.2000 |
| Sig. | | .178 | .068 | 1.000 |

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = .114.

**Descriptive Statistics** of the Hematology parameters of C. gariepinus exposed to varying concentration of **Antifouling paint.**

**Descriptives**

| | | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound | | |
| PCV | **CONTROL** | 3 | **13.7000** | .81854 | .47258 | 11.6666 | 15.7334 | 13.00 | 14.60 |
| | **7.5mg/L** | 3 | **13.5333** | .61101 | .35277 | 12.0155 | 15.0512 | 13.00 | 14.20 |
| | **10.0mg/L** | 3 | **10.5667** | .40415 | .23333 | 9.5627 | 11.5706 | 10.20 | 11.00 |
| | 12.5mg/L | 3 | **9.8333** | .15275 | .08819 | 9.4539 | 10.2128 | 9.70 | 10.00 |
| | 15mg/L | 3 | **8.6667** | .41633 | .24037 | 7.6324 | 9.7009 | 8.20 | 9.00 |
| | Total | 15 | 11.2600 | 2.13635 | .55160 | 10.0769 | 12.4431 | 8.20 | 14.60 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| WBC | CONTROL | 3 | 40.0333 | 1.95021 | 1.12596 | 35.1887 | 44.8779 | 38.10 | 42.00 |
| | 7.5mg/L | 3 | 54.1667 | 1.15902 | .66916 | 51.2875 | 57.0458 | 53.10 | 55.40 |
| | 10.0mg/L | 3 | 56.6333 | 1.38684 | .80069 | 53.1882 | 60.0784 | 55.10 | 57.80 |
| | 12.5mg/L | 3 | 71.2333 | 2.55799 | 1.47686 | 64.8789 | 77.5877 | 68.80 | 73.90 |
| | 15mg/L | 3 | 1.0210E2 | 3.35112 | 1.93477 | 93.7754 | 110.4246 | 98.80 | 105.50 |
| | Total | 15 | 64.8333 | 21.92056 | 5.65986 | 52.6941 | 76.9725 | 38.10 | 105.50 |
| RBC | CONTROL | 3 | 1.1033 | .02517 | .01453 | 1.0408 | 1.1658 | 1.08 | 1.13 |
| | 7.5mg/L | 3 | 1.0333 | .11372 | .06566 | .7508 | 1.3158 | .94 | 1.16 |
| | 10.0mg/L | 3 | .9533 | .05686 | .03283 | .8121 | 1.0946 | .89 | 1.00 |
| | 12.5mg/L | 3 | .9033 | .02517 | .01453 | .8408 | .9658 | .88 | .93 |
| | 15mg/L | 3 | .8467 | .02517 | .01453 | .7842 | .9092 | .82 | .87 |
| | Total | 15 | .9680 | .10732 | .02771 | .9086 | 1.0274 | .82 | 1.16 |
| NEUTROPHIL | CONTROL | 3 | 1.6667 | 1.15470 | .66667 | -1.2018 | 4.5351 | 1.00 | 3.00 |
| | 7.5mg/L | 3 | 1.0000 | .00000 | .00000 | 1.0000 | 1.0000 | 1.00 | 1.00 |
| | 10.0mg/L | 3 | .6667 | .57735 | .33333 | -.7676 | 2.1009 | .00 | 1.00 |
| | 12.5mg/L | 3 | .6667 | .57735 | .33333 | -.7676 | 2.1009 | .00 | 1.00 |
| | 15mg/L | 3 | .0000 | .00000 | .00000 | .0000 | .0000 | .00 | .00 |
| | Total | 15 | .8000 | .77460 | .20000 | .3710 | 1.2290 | .00 | 3.00 |
| EOSINOPHIL | CONTROL | 3 | .0000 | .00000 | .00000 | .0000 | .0000 | .00 | .00 |
| | 7.5mg/L | 3 | .0000 | .00000 | .00000 | .0000 | .0000 | .00 | .00 |
| | 10.0mg/L | 3 | .0000 | .00000 | .00000 | .0000 | .0000 | .00 | .00 |
| | 12.5mg/L | 3 | .0000 | .00000 | .00000 | .0000 | .0000 | .00 | .00 |
| | 15mg/L | 3 | .0000 | .00000 | .00000 | .0000 | .0000 | .00 | .00 |

|  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|
|  | Total | 15 | .0000 | .00000 | .00000 | .0000 | .0000 | .00 | .00 |
| BASOPHIL | CONTROL | 3 | .0000 | .00000 | .00000 | .0000 | .0000 | .00 | .00 |
|  | 7.5mg/L | 3 | .0000 | .00000 | .00000 | .0000 | .0000 | .00 | .00 |
|  | 10.0mg/L | 3 | .0000 | .00000 | .00000 | .0000 | .0000 | .00 | .00 |
|  | 12.5mg/L | 3 | .0000 | .00000 | .00000 | .0000 | .0000 | .00 | .00 |
|  | 15mg/L | 3 | .0000 | .00000 | .00000 | .0000 | .0000 | .00 | .00 |
|  | Total | 15 | .0000 | .00000 | .00000 | .0000 | .0000 | .00 | .00 |
| LYMPHOCYTE | CONTROL | 3 | 93.3333 | 3.05505 | 1.76383 | 85.7442 | 100.9225 | 90.00 | 96.00 |
|  | 7.5mg/L | 3 | 97.0000 | 1.00000 | .57735 | 94.5159 | 99.4841 | 96.00 | 98.00 |
|  | 10.0mg/L | 3 | 96.3333 | 1.52753 | .88192 | 92.5388 | 100.1279 | 95.00 | 98.00 |
|  | 12.5mg/L | 3 | 98.0000 | 1.00000 | .57735 | 95.5159 | 100.4841 | 97.00 | 99.00 |
|  | 15mg/L | 3 | 97.0000 | 2.00000 | 1.15470 | 92.0317 | 101.9683 | 95.00 | 99.00 |
|  | Total | 15 | 96.3333 | 2.28869 | .59094 | 95.0659 | 97.6008 | 90.00 | 99.00 |
| MONOCYTE | CONTROL | 3 | 2.6667 | .57735 | .33333 | 1.2324 | 4.1009 | 2.00 | 3.00 |
|  | 7.5mg/L | 3 | 2.0000 | 1.00000 | .57735 | -.4841 | 4.4841 | 1.00 | 3.00 |
|  | 10.0mg/L | 3 | .6667 | .57735 | .33333 | -.7676 | 2.1009 | .00 | 1.00 |
|  | 12.5mg/L | 3 | .6667 | .57735 | .33333 | -.7676 | 2.1009 | .00 | 1.00 |
|  | 15mg/L | 3 | 1.0000 | .00000 | .00000 | 1.0000 | 1.0000 | 1.00 | 1.00 |
|  | Total | 15 | 1.4000 | .98561 | .25448 | .8542 | 1.9458 | .00 | 3.00 |

**ANOVA**

|  | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| PCV | Between Groups | 61.089 | 4 | 15.272 | 54.414 | .000 |
| | Within Groups | 2.807 | 10 | .281 | | |
| | Total | 63.896 | 14 | | | |
| WBC | Between Groups | 6677.467 | 4 | 1669.367 | 335.979 | .000 |
| | Within Groups | 49.687 | 10 | 4.969 | | |
| | Total | 6727.153 | 14 | | | |
| RBC | Between Groups | .125 | 4 | .031 | 8.656 | .003 |
| | Within Groups | .036 | 10 | .004 | | |
| | Total | .161 | 14 | | | |
| NEUTROPHIL | Between Groups | 4.400 | 4 | 1.100 | 2.750 | .089 |
| | Within Groups | 4.000 | 10 | .400 | | |
| | Total | 8.400 | 14 | | | |
| EOSINOPHIL | Between Groups | .000 | 4 | .000 | . | . |
| | Within Groups | .000 | 10 | .000 | | |
| | Total | .000 | 14 | | | |
| BASOPHIL | Between Groups | .000 | 4 | .000 | . | . |
| | Within Groups | .000 | 10 | .000 | | |
| | Total | .000 | 14 | | | |
| LYMPHOCYTE | Between Groups | 38.000 | 4 | 9.500 | 2.689 | .093 |
| | Within Groups | 35.333 | 10 | 3.533 | | |
| | Total | 73.333 | 14 | | | |
| MONOCYTE | Between Groups | 9.600 | 4 | 2.400 | 6.000 | .010 |
| | Within Groups | 4.000 | 10 | .400 | | |
| | Total | 13.600 | 14 | | | |

**Post Hoc Tests**

**Homogeneous Subsets**

**PCV**

Duncan

| TREATMENT | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 15mg/L | 3 | 8.6667 | | |
| 12.5mg/L | 3 | | 9.8333 | |
| 10.0mg/L | 3 | | 10.5667 | |
| 7.5mg/L | 3 | | | 13.5333 |
| CONTROL | 3 | | | 13.7000 |
| Sig. | | 1.000 | .121 | .708 |

**Means** for groups in homogeneous subsets are **displayed.**

**WBC**

**Duncan**

| TREATMENT | N | Subset for alpha = 0.05 | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| **CONTROL** | 3 | 40.0333 | | | |
| **7.5mg/L** | 3 | | 54.1667 | | |
| **10.0mg/L** | 3 | | 56.6333 | | |
| **12.5mg/L** | 3 | | | 71.2333 | |
| **15mg/L** | 3 | | | | 1.0210E2 |
| **Sig.** | | 1.000 | .205 | 1.000 | 1.000 |

**Means for groups in homogeneous** subsets are displayed.

**NEUTROPHIL**

Duncan

| TREATMENT | N | Subset for alpha = 0.05 | |
|---|---|---|---|
| | | 1 | 2 |
| 15mg/L | 3 | .0000 | |
| 10.0mg/L | 3 | .6667 | .6667 |

**RBC**

**Duncan**

| TREATMENT | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 15mg/L | 3 | .8467 | | |
| 12.5mg/L | 3 | .9033 | | |
| 10.0mg/L | 3 | .9533 | .9533 | |
| 7.5mg/L | 3 | | 1.0333 | 1.0333 |
| CONTROL | 3 | | | 1.1033 |
| Sig. | | .064 | .134 | .184 |

Means for groups in homogeneous subsets are displayed.

**LYMPHOCYTE**

Duncan

| TREATMENT | N | Subset for alpha = 0.05 | |
|---|---|---|---|
| | | 1 | 2 |
| CONTROL | 3 | 93.3333 | |
| 10.0mg/L | 3 | 96.3333 | 96.3333 |
| 7.5mg/L | 3 | | 97.0000 |
| 15mg/L | 3 | | 97.0000 |
| 12.5mg/L | 3 | | 98.0000 |
| Sig. | | .079 | .335 |

Means for groups in homogeneous subsets are displayed.

**MONOCYTE**

**Duncan**

| TREATM | N | Subset for alpha = 0.05 |
|---|---|---|

| | | | |
|---|---|---|---|
| 12.5mg/L | 3 | .6667 | .6667 |
| 7.5mg/L | 3 | 1.0000 | 1.0000 |
| CONTROL | 3 | | 1.6667 |
| Sig. | | .101 | .101 |

Means for groups in homogeneous subsets are displayed.

| ENT | | 1 | 2 | 3 |
|---|---|---|---|---|
| 10.0mg/L | 3 | .6667 | | |
| 12.5mg/L | 3 | .6667 | | |
| 15mg/L | 3 | 1.0000 | 1.0000 | |
| 7.5mg/L | 3 | | 2.0000 | 2.0000 |
| CONTROL | 3 | | | 2.6667 |
| Sig. | | .552 | .082 | .226 |

Means for groups in homogeneous subsets are displayed.

**Descriptive Statistics of Heavy metal in water of *C. gariepinus* exposed to varying concentrations of Antifouling paint**

**Descriptives**

| | | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound | | |
| Cu | 7.5mg/L | 3 | .6533 | .02082 | .01202 | .6016 | .7050 | .63 | .67 |
| | 10.0mg/L | 3 | .8500 | .07937 | .04583 | .6528 | 1.0472 | .76 | .91 |
| | 12.5mg/L | 3 | 1.4900 | .08185 | .04726 | 1.2867 | 1.6933 | 1.40 | 1.56 |
| | 15.0mg/L | 3 | 1.9467 | .10214 | .05897 | 1.6929 | 2.2004 | 1.83 | 2.02 |
| | Total | 12 | 1.2350 | .54120 | .15623 | .8911 | 1.5789 | .63 | 2.02 |
| Zn | 7.5mg/L | 3 | 1.1933E2 | 10.69268 | 6.17342 | 92.7713 | 145.8954 | 110.00 | 131.00 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 10.0mg/L | 3 | 1.3233E2 | 9.07377 | 5.23874 | 109.7928 | 154.8738 | 122.00 | 139.00 |
| | 12.5mg/L | 3 | 1.5467E2 | 4.50925 | 2.60342 | 143.4651 | 165.8683 | 150.00 | 159.00 |
| | 15.0mg/L | 3 | 1.8067E2 | 7.50555 | 4.33333 | 162.0218 | 199.3115 | 173.00 | 188.00 |
| | Total | 12 | 1.4675E2 | 25.34175 | 7.31553 | 130.6486 | 162.8514 | 110.00 | 188.00 |
| Pb | 7.5mg/L | 3 | .0277 | .00306 | .00176 | .0201 | .0353 | .02 | .03 |
| | 10.0mg/L | 3 | .0493 | .01447 | .00835 | .0134 | .0853 | .04 | .07 |
| | 12.5mg/L | 3 | .0767 | .02079 | .01200 | .0250 | .1283 | .05 | .09 |
| | 15.0mg/L | 3 | .4183 | .49559 | .28613 | -.8128 | 1.6494 | .11 | .99 |
| | Total | 12 | .1430 | .26957 | .07782 | -.0283 | .3143 | .02 | .99 |
| Fe | 7.5mg/L | 3 | .1077 | .00681 | .00393 | .0908 | .1246 | .10 | .11 |
| | 10.0mg/L | 3 | .1250 | .00265 | .00153 | .1184 | .1316 | .12 | .13 |
| | 12.5mg/L | 3 | .2433 | .08505 | .04910 | .0321 | .4546 | .18 | .34 |
| | 15.0mg/L | 3 | .3700 | .04000 | .02309 | .2706 | .4694 | .33 | .41 |
| | Total | 12 | .2115 | .11715 | .03382 | .1371 | .2859 | .10 | .41 |

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Cu | Between Groups | 3.174 | 3 | 1.058 | 177.328 | .000 |
| | Within Groups | .048 | 8 | .006 | | |
| | Total | 3.222 | 11 | | | |
| Zn | Between Groups | 6517.583 | 3 | 2172.528 | 31.793 | .000 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Within Groups | 546.667 | 8 | 68.333 | | |
| | Total | 7064.250 | 11 | | | |
| Pb | Between Groups | .307 | 3 | .102 | 1.661 | .251 |
| | Within Groups | .493 | 8 | .062 | | |
| | Total | .799 | 11 | | | |
| Fe | Between Groups | .133 | 3 | .044 | 19.985 | .000 |
| | Within Groups | .018 | 8 | .002 | | |
| | Total | .151 | 11 | | | |

**Post Hoc Tests**

**Homogeneous Subsets**

**Cu**

Duncan

| Treatment | N | Subset for alpha = 0.05 | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| 7.5mg/L | 3 | .6533 | | | |
| 10.0mg/L | 3 | | .8500 | | |
| 12.5mg/L | 3 | | | 1.4900 | |
| 15.0mg/L | 3 | | | | 1.9467 |
| Sig. | | 1.000 | 1.000 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.

**Zn**

Duncan

| Treatment | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 7.5mg/L | 3 | 1.1933E2 | | |
| 10.0mg/L | 3 | 1.3233E2 | | |

| | | | | |
|---|---|---|---|---|
| 12.5mg/L | 3 | | 1.5467E2 | |
| 15.0mg/L | 3 | | | 1.8067E2 |
| Sig. | | .090 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.

**Pb**

Duncan

| Treatment | N | Subset for alpha = 0.05 |
|---|---|---|
| | | 1 |
| 7.5mg/L | 3 | .0277 |
| 10.0mg/L | 3 | .0493 |
| 12.5mg/L | 3 | .0767 |
| 15.0mg/L | 3 | .4183 |
| Sig. | | .108 |

Means for groups in homogeneous subsets are displayed.

**Fe**

Duncan

| Treatment | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 7.5mg/L | 3 | .1077 | | |
| 10.0mg/L | 3 | .1250 | | |
| 12.5mg/L | 3 | | .2433 | |
| 15.0mg/L | 3 | | | .3700 |
| Sig. | | .664 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.

**Descriptive Statistics of Heavy metal in fish of *C. gariepinus* exposed to varying concentrations of Antifouling paint**

**Descriptives**

| | | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound | | |
| Cu | 7.5mg/L | 3 | .3900 | .07211 | .04163 | .2109 | .5691 | .31 | .45 |
| | 10.0mg/L | 3 | .7233 | .09018 | .05207 | .4993 | .9474 | .63 | .81 |
| | 12.5mg/L | 3 | 1.1067 | .24786 | .14310 | .4910 | 1.7224 | .84 | 1.33 |
| | 15.0mg/L | 3 | 1.2467 | .04163 | .02404 | 1.1432 | 1.3501 | 1.20 | 1.28 |
| | Total | 12 | .8667 | .36955 | .10668 | .6319 | 1.1015 | .31 | 1.33 |
| Zn | 7.5mg/L | 3 | 95.6667 | 4.04145 | 2.33333 | 85.6271 | 105.7062 | 92.00 | 100.00 |
| | 10.0mg/L | 3 | 1.1233E2 | 10.78579 | 6.22718 | 85.5399 | 139.1267 | 100.00 | 120.00 |
| | 12.5mg/L | 3 | 1.2967E2 | 8.38650 | 4.84195 | 108.8335 | 150.4999 | 120.00 | 135.00 |
| | 15.0mg/L | 3 | 1.5533E2 | 4.50925 | 2.60342 | 144.1317 | 166.5349 | 151.00 | 160.00 |
| | Total | 12 | 1.2325E2 | 23.92840 | 6.90753 | 108.0466 | 138.4534 | 92.00 | 160.00 |
| Pb | 7.5mg/L | 3 | .0053 | .00252 | .00145 | -.0009 | .0116 | .00 | .01 |
| | 10.0mg/L | 3 | .0083 | .00115 | .00067 | .0055 | .0112 | .01 | .01 |
| | 12.5mg/L | 3 | .0260 | .00557 | .00321 | .0122 | .0398 | .02 | .03 |
| | 15.0mg/L | 3 | .0690 | .00917 | .00529 | .0462 | .0918 | .06 | .08 |
| | Total | 12 | .0272 | .02696 | .00778 | .0100 | .0443 | .00 | .08 |
| Fe | 7.5mg/L | 3 | .0717 | .01815 | .01048 | .0266 | .1167 | .06 | .09 |

| | N | Mean | Std. Deviation | Std. Error | Lower Bound | Upper Bound | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| 10.0mg/L | 3 | .0840 | .00889 | .00513 | .0619 | .1061 | .07 | .09 |
| 12.5mg/L | 3 | .1173 | .00351 | .00203 | .1086 | .1261 | .11 | .12 |
| 15.0mg/L | 3 | .2287 | .02074 | .01198 | .1771 | .2802 | .21 | .25 |
| Total | 12 | .1254 | .06585 | .01901 | .0836 | .1673 | .06 | .25 |

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Cu | Between Groups | 1.349 | 3 | .450 | 23.517 | .000 |
| | Within Groups | .153 | 8 | .019 | | |
| | Total | 1.502 | 11 | | | |
| Zn | Between Groups | 5851.583 | 3 | 1950.528 | 34.935 | .000 |
| | Within Groups | 446.667 | 8 | 55.833 | | |
| | Total | 6298.250 | 11 | | | |
| Pb | Between Groups | .008 | 3 | .003 | 84.221 | .000 |
| | Within Groups | .000 | 8 | .000 | | |
| | Total | .008 | 11 | | | |
| Fe | Between Groups | .046 | 3 | .015 | 72.058 | .000 |
| | Within Groups | .002 | 8 | .000 | | |
| | Total | .048 | 11 | | | |

**Post Hoc Tests**

**Homogeneous Subsets**

**Cu**

Duncan

| TREATMENT | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 7.5mg/L | 3 | .3900 | | |
| 10.0mg/L | 3 | | .7233 | |
| 12.5mg/L | 3 | | | 1.1067 |
| 15.0mg/L | 3 | | | 1.2467 |
| Sig. | | 1.000 | 1.000 | .250 |

Means for groups in homogeneous subsets are displayed.

**Zn**

Duncan

| TREATMENT | N | Subset for alpha = 0.05 | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| 7.5mg/L | 3 | 95.6667 | | | |
| 10.0mg/L | 3 | | 1.1233E2 | | |
| 12.5mg/L | 3 | | | 1.2967E2 | |
| 15.0mg/L | 3 | | | | 1.5533E2 |
| Sig. | | 1.000 | 1.000 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.

**Pb**

Duncan

| TREATMENT | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 7.5mg/L | 3 | .0053 | | |
| 10.0mg/L | 3 | .0083 | | |
| 12.5mg/L | 3 | | .0260 | |
| 15.0mg/L | 3 | | | .0690 |
| Sig. | | .526 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.

**Fe**

Duncan

| TREATMENT | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 7.5mg/L | 3 | .0717 | | |
| 10.0mg/L | 3 | .0840 | | |
| 12.5mg/L | 3 | | .1173 | |
| 15.0mg/L | 3 | | | .2287 |
| Sig. | | .331 | 1.000 | 1.000 |

Means for groups in homogeneous subsets are displayed.